Pro gradu –tutkielma

Effects of temperature and feeding regime on compensatory growth of rainbow trout, *Oncorhynchus mykiss*

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TIIVISTELMÄ

Kokeessa tutkittiin lämpötilan ja paastojakson pituuden vaikutusta kirjolohen, *Oncorhynchus mykiss*, kasvuun ja morfologiaan. Kokeen alussa 30 kpl 0+ -ikäistä kalaa (18.56–42.99g) jaettiin satunnaisesti kahteen eri lämpötilakäsittelyyn (17 °C ja 20.5 °C) ja kolmeen eri ruokintakäsittelyyn (*ad libitum* ja kolmen tai kuuden vuorokauden paasto ruokintojen välillä). Ruokintasyklejä pidettiin yllä 56 vrk, jonka aikana kalat punnittiin ja mitattiin viikoittain. Kokeen lopussa kalat tapettiin, ja sisäelimetön ruho, viskeraalirasvakudos, maksa, mahalaukku sekä mahalaukun sisältö punnittiin. Ruokintojen välillä kolme päivää paastotettujen kirjolohien paino ja pituus kokeen lopussa eivät eronneet merkitsevästi kontrollikalojen painosta tai pituudesta kummassakaan käsittelylämpötilassa, joten kalat osoittivat kompensaatiokasvua kokeen aikana. Myös kalat, joita oli paastotettu kuusi päivää ruokintojen välillä, kykenivät kompensoimaan täysin 20.5 °C:ssa, mutta osoittivat vain osittaista kasvun kompensaatiota 17 °C:ssa. Paastotetut kirjolohet kompensoivat lisääntyneen ravinnonoton avulla; ravinnonkäyttötehokkuus (FE) sen sijaan ei kokeen aikana eronnut eri ruokintakäsittelyjen välillä. Paastotettujen kalojen spesifinen kasvunopeus (SGR) ruokinnan aikana oli suurempi kuin kontrolliryhmien kaloilla. Paasto-ruokinta –käsittely ei aiheuttanut merkittäviä muutoksia kalojen morfologiassa, mutta viskeraalirasvakudoksen määrä oli kontrollikaloilla merkitsevästi korkeampi. Myös lämpötilalla oli vaikutus viskeraalirasvaprosenttiin, joka oli korkeampi 20.5 °C:ssa kaikilla ruokintakäsittelyillä. Kokeessa oli melko paljon yksilöiden välisiä eroja käsittelyjen sisällä, mutta tuloksista voidaan silti päätellä, että kirjolohet pystyvät hyvin toipumaan kohtuullisen pituisesta paastosta, ja että toipuminen on mahdollista myös optimilämpötilaa lämpimämmässäkin vedessä. Paasto-ruokinta –käsittelyä voitaisiin soveltaa kalankasvattamoilla, jos tarvetta on joustavampaan aikatauluun kalojen ruokinnassa ja vähempiin ruokintakertoihin. Myös tuotantoa ja tehokkuutta kirjolohen viljelyssä voitaisiin lisätä ja rehujätteen määrää taas vähentää löytämällä optimaaliset viljelyolosuhteet. Ylijäämärehun määrän väheneminen vuorostaan johtaisi kalankasvattamoilta ympäristöön huuhtoutuvien typpi- ja fosforipäästöjen pienenemiseen sekä ympäristölle koituvien haittojen vähenemiseen.

ABSTRACT

Feeding regime of *ad libitum* feeding or three or six days of deprivation between feeding periods was randomly assigned to 30 juvenile rainbow trout, *Oncorhynchus mykiss*, (18.56 - 42.99 g) held at two different temperatures, 17 °C and 20.5 °C. Cycles of feeding and deprivation were continued for 56 days, and weight and length of fish were measured weekly. At the termination of the experiment, the fish were killed, eviscerated, and weight of carcass, visceral fat, liver, stomach and stomach contents were recorded. Rainbow trout starved for three days between feedings and held at 17 °C and both food deprived groups at 20.5 °C were able to show full growth compensation and reached similar weight and length as the control fish at the end of the experiment. Fish intermittently starved for six days at 17 °C, on the other hand, could show only partial growth compensation and their weight and length differed significantly from the control group. The fish were able to compensate trough increased food consumption rather than increased feed efficiency (FE), and showed greater specific growth rate (SGR) during feeding. Intermittent feeding regime did not cause severe morphological changes, but the amount of visceral fat was higher in control fish compared to the starvation groups. Also temperature had an effect on the visceral fat percentage, which was higher among all the feeding groups held at 20.5 °C. The results in the present experiment were somewhat masked by relatively large inter-individual variation, but indicated that rainbow trout are able to recover from moderate food deprivation, also when subjected to slightly higher temperature than optimal. Intermittent feeding regime could be introduced in fish farms if more flexible feeding schedule with fewer feeding events was desirable. Also by finding optimal conditions in rainbow trout culture the efficiency and productivity could be increased along with reduction in the amount of feed waste. Reducing feed waste could in turn help to reduce the leaching of nitrogenous and phosphorous material into the environment.

Contents

1. INTRODUCTION

Temperature is one of the main abiotic factors that control growth of poikilothermic organisms living in aquatic environment. The effect of ambient temperature on growth has been studied on various organisms, including many fish species. Generally, increasing the prevailing temperature leads to exponentially increasing growth up to a certain point after which growth starts to rapidly decrease (Jobling 1994). A species-specific temperature range where maximal growth occurs, defined as the optimum temperature of the organism, has been found for many fish species. The optimum temperature for growth of rainbow trout, *Oncorhynchus mykiss*, varies from 16.5 to 17.2 °C according to different authors (Jobling 1981). It has been suspected that the preferred and optimum temperature of rainbow trout varies slightly according to life stage, juvenile fish preferring a few degrees warmer ambient temperature than adult fish (Jobling 1981).

In nature, the amount and distribution of food or prey is another factor that has a great effect on growth of an organism. Distribution can vary over the time of day or year, food being often more scarce during the winter months (Jobling 1994). The patchy distribution of planktonic prey can cause difficulties in finding food, especially for juvenile fish, and inter- and intraspecific competition can cause further limitations to the food intake. Organisms have developed different ways in which they can cope with the limitations of food, one of which is the phenomenon known as compensatory growth. Compensatory or catch-up growth occurs throughout the animal kingdom and can be defined as a sudden growth spurt that usually occurs after a continued period of reduced food availability (Mommsen 1998). During compensation, organisms, such as fish, can grow faster than expected catching up for the lost growth and quickly reaching similar size as conspecifics that have been fed continuously (Mommsen 1998). Usually the individuals that are in the poorest condition show the greatest response after adequate food supplies are restored (Jobling 1994). Compensatory growth has been studied on various fishes, such as salmonids (e.g. Dobson & Holmes 1984, Maclean & Metcalfe 2001, Quinton & Blake 1990, Reimers *et al*. 1993), cyprinids (e.g. van Dijk *et al*. 2002, Wieser *et al*. 1992), percids (e.g. Hayward & Wang 2001), channel catfish, *Ictalurus punctatus* (e.g. Gaylord & Gatlin 2000), cod, *Gadus morhua* (e.g. Bélanger *et al*. 2002), hybrid sunfish, *Lepomis cyanellus* × *L. macrochirus* (e.g. Hayward *et al*. 1997, Hayward *et al*. 2000) and hybrid tilapia, *Oreochromis mossambicus* × *O. niloticus* (Wang *et al*. 2000).

The purpose of this study was to find out how growth of rainbow trout is affected by two different temperature treatments, 17 °C and 20.5 °C, and three different feeding regimes (continuous feeding and cycles of starvation of three or six days followed by refeeding). The role of compensatory growth and the effect of temperature and feeding regime on some physiological and morphological parameters were also examined. The fasting-refeeding regimes used in the present study were selected because a similar protocol induced growth overcompensation in juvenile hybrid sunfish *Lepomis cyanellus* × *L. macrochirus* (Hayward *et al*. 1997), which is the only case where overcompensation has been reported. The temperatures were chosen as optimum temperature for growth of rainbow trout (17 °C) and a few degrees higher temperature (20.5 °C) that is still relatively near to the optimal temperature range without being too close to the upper thermal tolerance limit of the species.

The present study brings further information of the compensatory growth phenomenon to the scientific field. In addition, finding optimal conditions in rainbow trout culture would lead to increased productivity in fish farms. Increasing productivity by increased feed efficiency with reduction in feed waste could in turn reduce the amount of leaching nutrients from fish farms to the environment. To my knowledge, there has been

only one experiment where both water temperature and food availability have been manipulated and the effects of the two parameters together on fish growth studied (experiment on roach, *Rutilus rutilus*, by van Dijk *et al.* 2002), but the present study is the first one to examine the effect of the two parameters on growth on a commercially important species. Furthermore, this is the first known experiment where growth of individually held fish subjected to different temperature and feeding regimes is monitored.

2. BACKGROUND

2.1. Effect of temperature on metabolism, growth and appetite of fish

Metabolic rate of fish is known to increase exponentially with increasing temperature (Jobling 1994). Briggs & Post (1997) measured the metabolic rate of rainbow trout in the field and found that it increased linearly with increasing temperature from 3 °C to 22 °C. Mallekh & Lagardère (2002) found in their experiment on turbot (*Scophthalamus maximus*) that with increasing temperature (6-22 °C) the standard metabolic rate of the fish increased exponentially along with a six fold increase in standard oxygen uptake.

Various studies have concentrated on the effects of temperature on fish growth. Silverstein *et al.* (2000) found that channel catfish grew faster and had better feed efficiency at 26 °C than at 21.7 °C. The optimum temperature for growth of most channel catfish strains is between 26-32 °C (Silverstein *et al.* 2000). Yearling Arctic cisco (*Coregonus autumnalils*) held at 10 °C showed better feed conversion efficiency compared to fish held at 5 °C (Fechhelm *et al.* 1993). Specific growth rate and food consumption of Atlantic halibut (*Hippoglossus hippoglossus*) were higher at 12 °C (near-optimum) than at 6 °C, which indicated an increase in metabolic rate with temperature in accordance with optimum temperature range (Jonassen *et al.* 2000).

Also the food consumption of fish increases with increasing temperature up to a certain point (Jobling 1994). The temperature where food consumption reaches its maximum level is slightly higher than optimum temperature for growth (Jobling 1994). Daily feeding rate of turbot increased linearly with increasing temperature from 12 °C to 18 °C (Mallekh & Lagardère 2002). However, food intake and consequently growth can be suppressed if water temperature is raised to levels that are approaching the upper thermal tolerance limit of the species. Dockray *et al.* (1996) found that the increase of fluctuating average summer temperature (13-24 °C) by 2 °C depressed appetite, gross conversion efficiency and growth of juvenile rainbow trout, especially when the temperature reached 26 °C. According to Kaya (1978), the ultimate upper lethal temperature of rainbow trout is around 26.2 °C. In general, the increase of 2 °C to the naturally fluctuating thermal cycle appears to be beneficial to rainbow trout until their optimum temperature for growth is surpassed (Dockray *et al.* 1996).

Fish are able to acclimate to wider temperature ranges, if the prevailing temperature is lowered or raised gradually. According to Jobling (1994), this acclimatization takes place more rapidly when the temperature is increasing rather than decreasing. Similar metabolic rates among rainbow trout living in normal and elevated (+2 °C) temperatures indicated that the fish were able to acclimate to the elevated temperature, especially when the increase in temperature occurred gradually along with daily fluctuations (Dockray *et al.* 1996).

Limitations in food supply can reduce growth if temperature is raised simultaneously. Continuous elevation in temperature reduced growth rates when rainbow

trout were fed limited (1 % of body weight · d^{-1}) food rations (Dockray *et al.* 1998). These authors suggested that with limited energy uptake the metabolic energy expenditure accounted for greater proportion of the overall energy budget, and the growth became increasingly sensitive to minor metabolic differences (such as the elevation of temperature by 2 °C). Russell *et al.* (1996) found that growth of bass (*Dicentrarchus labrax*) fed maximum rations increased with increasing temperature, but the temperature range used in their experiment (6-18 °C) was below the optimum temperature for the growth of bass (~ 22 °C). When given restricted rations, the growth rate of bass decreased with increasing temperature (Russell *et al.* 1996).

2.2. Feeding and compensatory growth

2.2.1. Effects of starvation

Fish can withstand relatively long periods of starvation without severe consequences. Starvation of 62 days did not cause increased mortality in second sea winter Atlantic salmon, *Salmo salar* (Reimers *et al.* 1993). Edsall *et al.* (2003) studied mortality of lake trout (*Salvelinus namaycush*) fry at two temperatures and found them to be highly resistant to death by starvation. The point of no return for 50 % mortality occurred after 52 days of starvation for fry at 7 °C and after 24 days at 12 °C. Bilton & Robbins (1973) found in their study on sockeye salmon fry (*O. nerka*), that the fish were capable of dealing with 3-4 weeks of starvation with less than 10 % mortality. However, even if the fish were able to survive from a period of starvation, there can still be some deleterious long-term side-effects, such as delayed maturation (Reimers *et al.* 1993) and increased susceptibility to predation due to smaller size (Edsall *et al.* 2003).

2.2.2. Why does growth compensation occur?

After starvation or food restriction fish are known to show greater growth rates than the constantly fed fish, when adequate food supplies are restored (e.g. Dobson & Holmes 1984, Miglavs & Jobling 1989a, Quinton & Blake 1990), in other words, show growth compensation. After spawning, the energy deposits of fish can be severely depleted, and during the post-spawning period these reserves will be rapidly replenished giving faster rates of weight gain (Jobling 1994). There are advantages in gaining a greater size, such as decreased mortality, increased fecundity and egg size and increased prey size selection (Ali *et al.* 2003). Holtby *et al.* (1990) found in their experiment on coho salmon (*O. kisutch*) smolts that marine survival was strongly correlated with early ocean growth, and that mortality was high during years when growth rates were particularly low. The ability to compensate for lost growth is thus an important adaptation in a fluctuating and unpredictable environment (Maclean & Metcalfe 2001).

However, there are also some disadvantages associated with compensatory growth. Increased search for food and more time spent on feeding can cause increased susceptibility to predators, increased aggressiveness and fighting for food demand energy and can cause injuries, and higher metabolic costs are associated with increased mortality and oxygen uptake (disadvantage if hypoxic conditions prevail) (Ali *et al.* 2003). Faster growth can cause developmental abnormalities and induce lower growth rates and lipid reserves in the longer term, and there is a trade-off between growth and swimming performance (Ali *et al.* 2003). In addition, Johnsson & Bohlin (2006) hypothesized that compensatory growth could occur at the cost of reduced immunocompetence and disease resistance.

2.2.3. Types of compensatory growth

There are several examples where fish have not fully compensated for the lost growth after a period of food restriction or deprivation. According to Miglavs & Jobling (1989b) Arctic charr (*Salvelinus alpinus*) subjected to food restriction followed by eight weeks of satiation feeding were significantly smaller than controls fed *ad libitum* at the end of the experiment and thus compensated only partially. Also Wang *et al*. (2000) found only partial compensation in hybrid tilapia that were deprived of food for 2-4 weeks before the refeeding period of four weeks, but on the other hand, the fish fasted for one week fully compensated their growth. The authors suggested that the failure of fish starved longer to fully compensate might be due to relatively weak capacity for compensatory growth of hybrid tilapia.

Full growth compensation has occurred in many studies. Quinton & Blake (1990) found that rainbow trout starved for three weeks and fed for another three weeks grew faster than the control fish. During the final week of refeeding the average percentage increase in growth was much greater than that found in constantly fed fish. Dobson & Holmes (1984) found that rainbow trout on a similar feeding schedule were able to compensate their growth to the level of continuously fed control fish, or over. They also noticed that the compensation was most effective when the water temperature increased during the summer months close to the optimum temperature for growth of rainbow trout (near 16 °C). Tian & Qin (2003) observed full compensation in barramundi, *Lates calcarifer*, starved for one week prior *ad libitum* feeding until week eight, but only partial compensation in fish that were deprived for two or three weeks. They hypothesized that if weight of the fish drops under 60% of the control fish weight during starvation, full compensation is unlikely to occur. In a later study, Tian & Qin (2004) suggested that "complete compensatory growth occurs only in fish experiencing moderate feed restriction, with more severe restrictions only resulting in a partial compensatory response". They found in their study on barramundi that the fish fed at 50% and 75% satiation caught up with the *ad libitum* fed control fish weight, but the fish fed at 0% or 25% did not. Nikki *et al*. (2004) found in their experiment on rainbow trout held individually that the end weight of fish starved in cycles for two or four days did not differ from the weight of the continuously fed control fish, and that at the end of the experiment the specific growth rate of fish starved in cycles for eight or 14 days was significantly higher compared to the control fish. They also hypothesized that in order to fully compensate the fish must get used to the intermittent feeding regime, and that the feeding period must be long enough compared to the number of fasting days in order for a full recovery to occur. Full growth compensation was observed also by Miglavs & Jobling (1989a) in their study on arctic charr, Xie *et al*. (2001) in gibel carp, *Carassius auratus gibelio*, and Skilbrei (1990) in Atlantic salmon male parr.

There is one study in which the previously starved fish have overgrown the *ad libitum* fed control fish, a phenomenon called growth overcompensation (GOC). Hayward *et al*. (1997) observed overcompensation in juvenile hybrid sunfish fasted in cycles for two and 14 days and fed until their food consumption no longer exceeded the levels of the continuously fed control fish. However, GOC was not observed if the sunfish hybrids were reared in groups (Hayward *et al*. 2000). Hayward & Wang (2001) also failed to induce overcompensation in individually held yellow perch (*Perca flavescens*) when the fish were held on a similar feeding regime, but observed only partial compensation. On the other hand, when food deprivation of 12 days was replaced by maintenance feeding for 12 days, the yellow perch showed full compensation in growth during the first few feeding cycles. After the few feeding cycles of rapid growth, the growth of food restricted yellow perch

slowed down once the fish reached the mass of the controls. Hayward & Wang (2001) suggested that maturing yellow perch lacked growth overcompensating capacity, and that in order for growth overcompensation to occur, the growth limiting mechanism that was present in maturing yellow perch must be absent or less restrictive. They also suggested that GOC may be species specific, or limited to certain life stage occurring in juvenile but not in maturing or mature fish. According to them, it is reasonable to expect that GOC capacity would not exist in any life stages of fish that have evolved in highly stable environment where individuals rarely experience poor growth conditions.

Weatherley & Gill (1981) discovered in their experiment on rainbow trout, that the fish that had been starved for the longest time (13 weeks) and then refed, were able to grow faster than the controls and tended to reach greater dry weight compared to the controls during recovery growth. Jobling *et al*. (1993) found in their study on Arctic charr held in constant (9-10.5 °C) temperature that the fish that had been starved and refed in cycles, were significantly heavier than continuously fed control fish when the weights were compared after equal number of feeding days. The fish that had the longest starvation-feeding cycle (three weeks of starvation followed by three weeks of feeding) had the highest specific growth rate when compared to other feeding groups. These findings support Russell & Wootton (1992) who suggested that compensatory response reflects the length of the prior starvation, but are at least somewhat contradictory to the results obtained by Tian & Qin (2003) who hypothesized that if the weight of the fish drops to too low levels due to longer period of starvation, full compensation does not occur.

2.2.4. Mechanisms behind compensatory growth

Metabolic rates decrease in starved fish (Jobling 1994, Wieser *et al*. 1992). Starved cyprinids were saving energy by reducing locomotory activity and possibly also by reducing the cost of maintenance functions in a study by Wieser *et al*. (1992). It has been suggested that when fish are transferred from starvation or restricted feeding to satiation feeding, the metabolic rate does not immediately return to the level of the continuously fed fish. Low maintenance costs added with high food and energy uptake would lead to large amount of energy being available for growth. Thus, weight gain would be rapid during the initial phase of recovery period. It is also possible that catabolic processes slow down while anabolic processes are accelerated, and this causes the rapid growth rates during compensatory phase (Jobling 1994).

In most studies where growth compensation has been observed the fish have become hyperphagic during the refeeding period. The food deprived hybrid sunfish consumed significantly more food upon refeeding than their *ad libitum* fed conspecifics (Hayward *et al*. 1997). According to Bull & Metcalfe (1997), juvenile Atlantic salmon parr became hyperphagic after a period of food deprivation during which they had lost some of their fat reserves. The extent of the energy loss seemed to affect primarily the duration of the hyperphagic response rather than its magnitude (intensity of feeding). The same phenomenon was observed by Ali & Wootton (2001) in their experiment on juvenile three-spined stickleback, *Gasterosteus aculeatus*.

In few experiments the food deprived fish have been able to compensate for lost growth by the means of increased conversion efficiency rather than becoming hyperphagic. Increase in weight gain in rainbow trout during a growth spurt was not an effect of increased appetite related to the duration of feed deprivation, but was caused by an increase in feed conversion efficiency (Boujard *et al*. 2000). Minnows (*Phoxinus phoxinus*) and sticklebacks starved for one or two weeks were hyperphagic during compensatory phase, but improved growth efficiency was observed only in the groups starved for two

weeks (Zhu *et al*. 2001). Also a combination of both mechanisms has resulted in growth compensation. According to Qian *et al*. (2000), the feed intake, conversion efficiency and protein and energy retention efficiency during re-alimentation period were significantly higher among the starved-refed gibel carp compared to the controls.

2.2.5. Control of compensatory growth

It has been suggested that rapid compensatory growth spurt occurs in response to an assessment of the ratio of storage tissues (fat deposits) to structural tissues (e.g. bone), known as the lipostat model (Jobling & Johansen 1999). The degree of reduction and repletion of fat stores of Atlantic salmon correlated with both the magnitude and duration of hyperphagic and growth responses during excess feeding (Johansen *et al*. 2001). Bull & Metcalfe (1997) found juvenile Atlantic salmon responding to reduction in fat reserves due to food restriction by increased feeding that resulted in restoration of body fat lost during deprivation. Jobling & Johansen (1999) further hypothesized that if lipid accumulation took place very slowly during catch-up growth, hyperphagia would continue for a prolonged time and changes in lipid:lean body mass (LBM) ratio would occur gradually, which in turn could lead to growth overcompensation.

However, the experiment by Tian & Qin (2003) did not provide evidence for the lipostat model, since the 1-week deprived fish stopped compensatory responses when their weight caught up with the controls, even when their ratio of lipid to LBM was still lower. Further, Xie *et al*. 2001 found that compensatory growth response of gibel carp did not terminate until the body weight reached control level even though the body fat:LBM ratios in the starved fish were restored to control levels earlier. Skilbrei (1990) found similar results in an experiment on Atlantic salmon, when the elevated growth rate of the food deprived fish slowed down when the fish reached approximately the same size as their siblings fed unrestricted rations. Maclean & Metcalfe (2001) suggested that compensatory growth could be "a response to an assessment of absolute body size in comparison with a target size for the time of year" rather than a response to lipostatic cues. Xie *et al*. (2001), on the other hand, proposed that there could be a role of fatty acid composition in determining compensatory growth and possibly some kind of hierarchy of control mechanisms.

2.2.6. Food restriction is not the only way to induce compensatory growth

A compensatory response can be induced also by lowering the temperature well below the optimum temperature range of the organism. Nicieza & Metcalfe (1997) found Atlantic salmon to compensate after a period of reduced food availability and also when water temperature was maintained at lower levels and then raised back to optimal levels. However, in the latter case the compensation in growth was delayed (probably due to time needed for acclimatization) and not as complete because growth losses were greater in the temperature manipulated group (greater amount in weight to compensate). Also Maclean & Metcalfe (2001) observed faster growth rate and compensation in juvenile Atlantic salmon subjected to cooler temperature (8.4 °C) for three weeks followed by 20 weeks in the same temperature as the controls (16.4 °C). The authors concluded that growth compensation after a period of abnormally low temperature without food restriction should not be regulated by the same mechanism as proposed in the lipostat model, since the fish should have not depleted their fat reserves.

Compensatory growth can also occur when fish are transferred from water with low dissolved oxygen content to normoxic conditions. Foss & Imsland (2002) found a clear compensatory effect in juvenile spotted wolfish, *Anarhichas minor*, first reared under low

dissolved oxygen and then returned to normal oxygen levels. The fish showed elevated growth rates and improved food conversion efficiency.

2.2.7. Differences in compensatory growth response between fish species

Studies have shown that there are different ways how different fish species respond to food deprivation and refeeding. Zhu *et al*. (2001) found that the compensatory response (increased food consumption) in sticklebacks was delayed compared to the response in minnows. The minnows compensated fully for one or two weeks of deprivation within one or two weeks of refeeding, but it took four weeks for sticklebacks to fully compensate for one week of starvation. Sticklebacks starved for two weeks were still compensating after three weeks of refeeding when the experiment was terminated. The sticklebacks showed higher growth rates than minnows when fed, but they also lost more weight during starvation (Zhu *et al*. 2001). Also Wieser *et al*. (1992) found a lag in the compensatory growth response in chub, *Leuciscus cephalus*, but not in Danube bleak, *Chalcalburnus chalcoides mento*, or rudd, *Scardinius erythrophthalmus*.

In a study using one deprivation-refeeding cycle on three-spined stickleback, minnow and gibel carp (Ali *et al*. 2001) the authors suggested that "there are intrinsic interspecific differences in the control of appetite and these are reflected in the different responses after a period of food deprivation." The minnows lost weight at a lower rate than the other two species during deprivation. Like in previous studies, there was a time lag in hyperphagic response of sticklebacks. The authors suggested that since sticklebacks have true stomach as opposed to cyprinid species, there might be a need for them to re-establish digestive processes in the stomach after food deprivation. But sunfish hybrids also have a stomach, and no apparent time lag was found in the experiment by Hayward *et al*. (1997).

Wang *et al*. (2000) have discussed the possibility that some species such as hybrid tilapia have weaker capacity for growth compensation than some other species. Hayward & Wang (2001) suggested that maturing yellow perch lacked growth overcompensating capacity that the hybrid sunfish possessed, which could be a result of evolving in a more stable environment without severe fluctuations in food availability.

Differences in compensatory growth capacity between populations of the same species inhabiting different geographic areas are also reported. Schultz *et al*. (2002) found that high-latitude Atlantic silverside (*Menidia menidia*), which is faster growing in nature, compensated more readily than low-latitude individuals. The high-latitude fish also had higher gross growth efficiency and consumed more food than the low-latitude fish (Schultz *et al*. 2002).

2.2.8. Compensatory growth experiments in nature

Feeding and compensatory growth experiments are usually done in controlled laboratory conditions. Food is provided for the fish during re-alimentation phase and following growth is recorded. Laboratory experiments do not provide much information about survival and growth of the fish in their natural habitats. With so much fluctuation in resources, is it even certain that starved fish are able to show growth compensation or even survive in natural conditions? So far, there has been only one manipulative field experiment concerning catch-up growth of fish. Johnsson & Bohlin (2006) captured wild brown trout, *Salmo trutta*, tagged them and starved them in laboratory for zero, two, three or four weeks. After starvation period, the trout were released in the wild at the end of May and recaptured after one, five and ten months. After one month, weight was restored in the 2-week starved fish, but not in other groups. After five months, partial compensation was

observed in all the remaining groups. Deprived groups had significantly lower recapture rates in April than the controls, which suggests that their over-winter mortality was increased (Johnsson & Bohlin 2006). The authors suggested that the reasons for elevated mortality could be reduced investment in the muscular development which leads to impaired locomotor performance, increased maintenance costs and reduced foraging efficiency, predator avoidance and competitive ability. In addition, compensatory growth might have occurred at the cost of reduced immunocompetence and disease resistance (Johnsson & Bohlin 2006).

3. MATERIALS & METHODS

The experiment was conducted between August 29th and November 7th, 2005, in the research laboratory of the Department of Biological and Environmental Science, University of Jyväskylä. The experiment was carried out on 0+ female rainbow trout raised in Konnevesi Research Station. The weight of the fish at the beginning of the experiment varied between 18.56 and 42.99 g, the average being 27.60 g. One week before starting the feeding experiment 30 rainbow trout were transferred from a larger holding tank into the experimental aquaria. The experimental aquaria were 15 l glass wall flow-through tanks divided in half with a black polyethene divider. The fish were held individually in the divided aquarium halves (7.5 l), and the sides of the aquaria were covered with white paper in order to prevent fish from seeing each other. A 5 mm hole was drilled into the divider in order to allow water to flow through, because water inlet located on the one end and outlet on the other end of the tank. The incoming fresh water from communal supply was aerated in separate aeration tanks before the water flowed into the experimental aquaria. In addition, aquarium air pumps (Mouse Air pump M-106) and air stones were used in each of the aquarium halves in order to provide adequate oxygen levels. Activated carbon filters (Atlas Filtri 10 µm, Limena, Italy) were used to remove chlorine and other possible potentially harmful chemicals from the water. The fish were subjected to 24L: 0D rhythm throughout the experiment. Constant light was sustained by two fluorescent tubes above each group of four tanks. Water flow was adjusted to approximately $0.5 \ l \cdot min^{-1}$. The experimental design is presented in the Appendix.

The rainbow trout were randomly selected into two different temperature treatments (optimum - ~17 °C and warm - ~20.5 °C) and into three different starvation-refeeding regimes, which were zero, three or six days of starvation between feedings. These treatments were designated as control, D3 and D6, respectively. In total there were thus six treatment groups with five fish in each group. During the acclimation period of one week the temperature was gradually raised from ambient (approximately 13 °C) to temperatures used in the experiment. Desired temperatures were maintained by using electronic temperature regulators (Ouman Finland OY, Kempele). Food intake and general well-being of the fish were monitored during the week of acclimation, and two fish (one of which died and one had a cut on its lateral side) were replaced with another individual.

Water temperature (°C) was measured twice a day during the experiment. Light intensity was measured at the center of each aquarium half on the water surface by using a photo-radiometer (Delta Ohm HD 9221, Padua, Italy), and it averaged around 2610 lux (varied from 1650 to 3500 lux depending on the location of the tank in relation to the fluorescent tubes). Oxygen level, which was monitored weekly using YSI oxygen meter (Model 55/12 FT, YSI Inc., Yellow Springs, Ohio, USA), varied between 7.9 and 9.8 mg $\cdot$ l^{-1} in optimum temperature and between 7.1 and 8.7 mg $\cdot l^{-1}$ in warm water. The pH of the incoming water was also monitored weekly and it varied from 7.6 to 7.8 at 17 °C and from

7.4 to 7.9 at 20.5 °C (Combo pH & EC, Hanna Instruments, USA). A total gas pressure meter (PT4 Tracker, Point Four Systems Inc., Richmond, BC, Canada) was used to verify appropriate gas saturation level.

During the experiment the control groups were hand-fed *ad libitum* twice a day (8.00 and 16.00). The fish were fed commercial pellets manufactured especially for juvenile salmonids (Raisio, 26.0% fat, 46.0% protein, 0.8% fiber, 8.0% ash, 1.15% phosphorous, total energy content 23.8 MJkg^{-1} according to manufacturer) of the size of 2.5 mm during the first twelve days and of the size of 3.5 mm during the rest of the experiment. Feeding was continued until the fish showed no interest when food pellets were dropped into the tank or they started to spit the pellets out. In order to assure that the fish were satisfied, some pellets were left on the bottom of the tank until the next feeding. At the beginning of the experiment the fish in groups D3 and D6 were deprived of food for three and six days, respectively, after which they were fed the same way as the fish in the control group. Before every feeding event uneaten pellets from the previous feeding were removed from the bottom of the tanks by siphoning and counted. The amount of feed given was weighed and weight of uneaten pellets (based on the number of removed pellets) was subtracted to give the total amount consumed by the fish. The weight of a single pellet was estimated by weighing six batches of 100 pellets, and dividing the weight by 100. The starvation groups D3 and D6 were fed until their food consumption relative to their body weight did not differ from the food consumption of the controls more than 10 % (calculated as the average of three previous consecutive feeding days), or until the duration of the feeding period was three times longer than the starvation period. Thus the fish in D3 group were fed for a maximum of nine days and the fish in D6 group for a maximum of 18 days. After every feeding period the fish in D3 and D6 groups were again deprived of food for three or six days, respectively.

The rainbow trout were weighed to the nearest 0.01 g and their total length was measured to the nearest 1 mm once a week (on Mondays) during the experiment. The fish were anaesthetized with 40 mg · l^{-1} clove oil : ethanol mixture (1:9) during the measurements. The anaesthetic bath was aerated. After the measurements the fish were placed back into their experimental tanks to recover.

The experiment lasted for 56 days (eight weeks), after which all the fish were fasted for four days and then fed in excess. One hour after feeding they were killed by a sharp blow on the head. The fish were weighed, dissected and eviscerated, and reweighed. The stomach was emptied by pressing the stomach contents out with fingers, and both the stomach and its contents were weighed separately. Visceral fat tissue and liver were separated and weighed. In addition, stomach, the stomach contents and liver were dried at 60 °C for three days and their dry weights recorded. The dry weight of stomach contents was used as an estimate for total volume of the stomach (Pirhonen & Koskela 2005).

Hepatosomatic index (HI, percentage liver wet weight of body wet weight) was calculated from the measured parameters, as well as liver moisture content:

$$\text{Liver moisture (\%)} = (1 - \text{Liver dry mass (g)} \cdot \text{Liver wet mass (g)}^{-1}) \cdot 100$$

Fulton's condition factor (K) was calculated by using the following formula

$$K = W \cdot L^{-3} \cdot 100,$$

where W = fish weight (g) and L = fish length (cm).

Growth of the fish was compared by calculating the specific growth rate (SGR, % d^{-1}) by using the formula

$$SGR \ (\% \ d^{-1}) = 100 \cdot (lnW_2 - lnW_1) \cdot t^{-1},$$

where W_1 and W_2 = fish weight (g) at the beginning and end of a growth period, respectively, and t = number of days passed.

Feed efficiency (FE) was calculated by using the formula

$$FE = \text{weight gain (g)} \cdot \text{amount of food (g) eaten}^{-1} \text{ during a time period.}$$

All statistical tests were performed by using SPSS for Windows, version 13.0. A single fish was considered as the independent experimental unit, even though two fish shared the same water inlet and outlet (aquariums divided in half). This dependence was not considered to affect the results of the experiment. A multivariate analysis of variance (MANOVA) was run on the data from the final measurements in order to examine the possible effect of the different temperature and feeding regimes on the measured parameters. No interaction between temperature and feeding regime was found, so the effect of feeding on the measured parameters was examined separately in the two temperatures. At 20.5 °C, the weights of stomach dry contents were log-transformed in order to avoid heteroscedasticity. The data in visceral fat percentage and condition factor data remained heteroscedastic despite the transformations, but were normally distributed, so the nontransformed data was included in the MANOVA. At 17 °C, all the data were homoscedastic and therefore no transformations were needed. LSD –test (Zar 1999) was used (when differences in the initial analysis were significant) to find the differences between treatments in final weight and length, weight gain, increase in length, carcass weight, SGR (over the whole 8-week experiment), total food intake during the experiment, visceral fat percentage and hepatosomatic index. Fish final weight had a significant effect on the visceral fat percentage at 17 °C, so it was included in the analysis as a covariate. The initial weight was taken as a covariate of carcass weight at 20.5 °C. The possible role of fish weight or length as a covariate was examined when considering every parameter, but it was not significant in any case but the previous ones.

Feed efficiency (FE) was calculated for the whole experiment, as well as for every week of the experiment. The values for weeks one, four, five and eight were excluded from the analysis (MANOVA) because the D3 and D6 groups were deprived of food for considerable periods of time during those weeks. LSD –test was used to test for the pairwise differences among different feeding groups, if there were significant differences in the initial analysis.

The effect of feeding regime on SGR during feeding was compared by using one-way ANOVA. SGR was calculated for only one feeding period when comparing D3 fish,

and for three periods when comparing D6 fish with the controls at 17 °C. At 20.5 °C, SGRs were calculated for two feeding periods when comparing D3 fish and the controls, and for three periods when comparing D6 fish and the controls. SGRs were not calculated for all the feeding periods because the weighing was done regularly once a week and were thus out of phase with the feeding periods.

Correlation between final weight of the fish and log-transformed weight of visceral fat was tested by using linear regression, as well as the dependence between weight gain and the amount of food consumed by the fish. Logarithmic regression was used when testing the relationship between the final weight of the fish and liver wet weight.

Four fish died during the experiment (13.3 %). The cause of death in all but one case remained unknown; all the fish that died were from different treatment groups and suffered no apparent signs of disease or parasitic infection.

4. RESULTS

4.1. Weight and length gain

The initial weight or length of the fish did not differ significantly between the feeding regimes at 17 °C (P=0.122 and P=0.126, respectively), or at 20.5 °C (P=0.942 and P=0.904, respectively, Table 1). However, at the end of the experiment, total weight and carcass weight of the control fish significantly exceeded the total and carcass weights of the D6 –fish at 17 °C (P=0.009 and P=0.007, respectively), but did not differ significantly from the values of D3 –group (P=0.094 and P=0.085). There were no significant differences in the final total and carcass weights between the intermittently starved D3- and D6- fish (P=0.227 and P=0.210) at 17 °C, or between any of the feeding groups at 20.5 °C (P=0.216 and P=0.219). Temperature had no significant effect on the total weight of the fish at the end of the experiment (P=0.870), or on the carcass weight (P=0.829). Overall weight gain of the rainbow trout during the experiment is presented in Fig. 1.

The controls were also significantly longer at the end of the experiment than the D6 - fish (P=0.013, Table 1) at 17 °C, but otherwise there were no differences in the final length in this temperature treatment. At 20.5 °C, the final length of the fish did not differ significantly between any of the feeding regimes (P=0.273). There were no differences in the final length between the two temperature treatments (P=0.585).

Table 1. Measured parameters at the end of an 8-week feeding experiment of 0+ rainbow trout, *O. mykiss*, held at two temperatures and under three feeding regimes; *ad libitum* feeding (control), starvation of three days between feedings (D3) and starvation of six days between feedings (D6). Given values are group averages ± S.D.

	~17 °C			~20.5 °C		
	Control (n=4)	D3 (n=4)	D6 (n=5)	Control (n=5)	D3 (n=4)	D6 (n=4)
Initial weight (g)	30.55 ±8.81	31.52 ±3.68	24.97 ±4.96	27.53 ±3.08	25.97 ±4.62	25.04 ±3.88
Initial length (cm)	13.82 ±1.08	13.96 ±0.76	13.18 ±0.70	13.46 ±0.42	13.28 ±0.70	13.08 ±0.71
Final weight (g)	162.11 A ±29.30	116.16 AB ±47.03	85.80 B ±28.06	147.95 ±61.86	107.60 ±35.77	94.56 ±10.04
Final length (cm)	22.70 A ±0.99	20.20 AB ±2.51	18.68 B ±2.08	21.56 ±3.01	19.45 ±2.16	18.98 ±0.55
Carcass weight (g)	128.05 A ±23.71	91.80 AB ±35.34	67.73 B ±20.96	116.76 ±45.32	83.28 ±29.43	76.30 ±8.91
Specific growth rate (% d^{-1})	3.18 A ±0.22	2.16 B ±0.83	2.14 B ±0.54	2.82 ±0.86	2.47 ±0.36	2.32 ±0.38
Feed efficiency (g · g^{-1})	1.28 ±0.13	1.07 ±0.31	1.22 ±0.16	1.02 ±0.29	1.20 ±0.14	1.11 ±0.10
Visceral fat %	5.16 A ±1.23	2.78 B ±0.99	3.07 AB ±0.81	5.98 A ±1.44	4.51 B ±0.24	3.52 B ±1.23
Stomach content (dw/g)	3.80 ±1.67	4.14 ±2.35	3.03 ±1.84	3.91 ±2.66	3.68 ±1.99	2.72 ±0.46
Stomach weight %	1.30 ±0.24	1.66 ±0.23	1.58 ±0.28	1.36 ±0.32	1.54 ±0.29	1.15 ±0.18
Hepatosomatic index	0.93 ±0.06	0.98 ±0.34	1.09 ±0.17	0.96 A ±0.22	1.25 B ±0.18	1.08 AB ±0.15
Liver moisture (%)	73.43 ±0.84	72.12 ±0.96	71.91 ±1.69	74.85 ±1.74	71.42 ±5.61	71.81 ±1.29
Condition factor	1.37 ±0.10	1.34 ±0.13	1.27 ±0.11	1.37 ±0.20	1.42 ±0.03	1.38 ±0.05

Capital letters A and B represent significant difference at <0.05 level (LSD Post-Hoc test) within a temperature treatment.
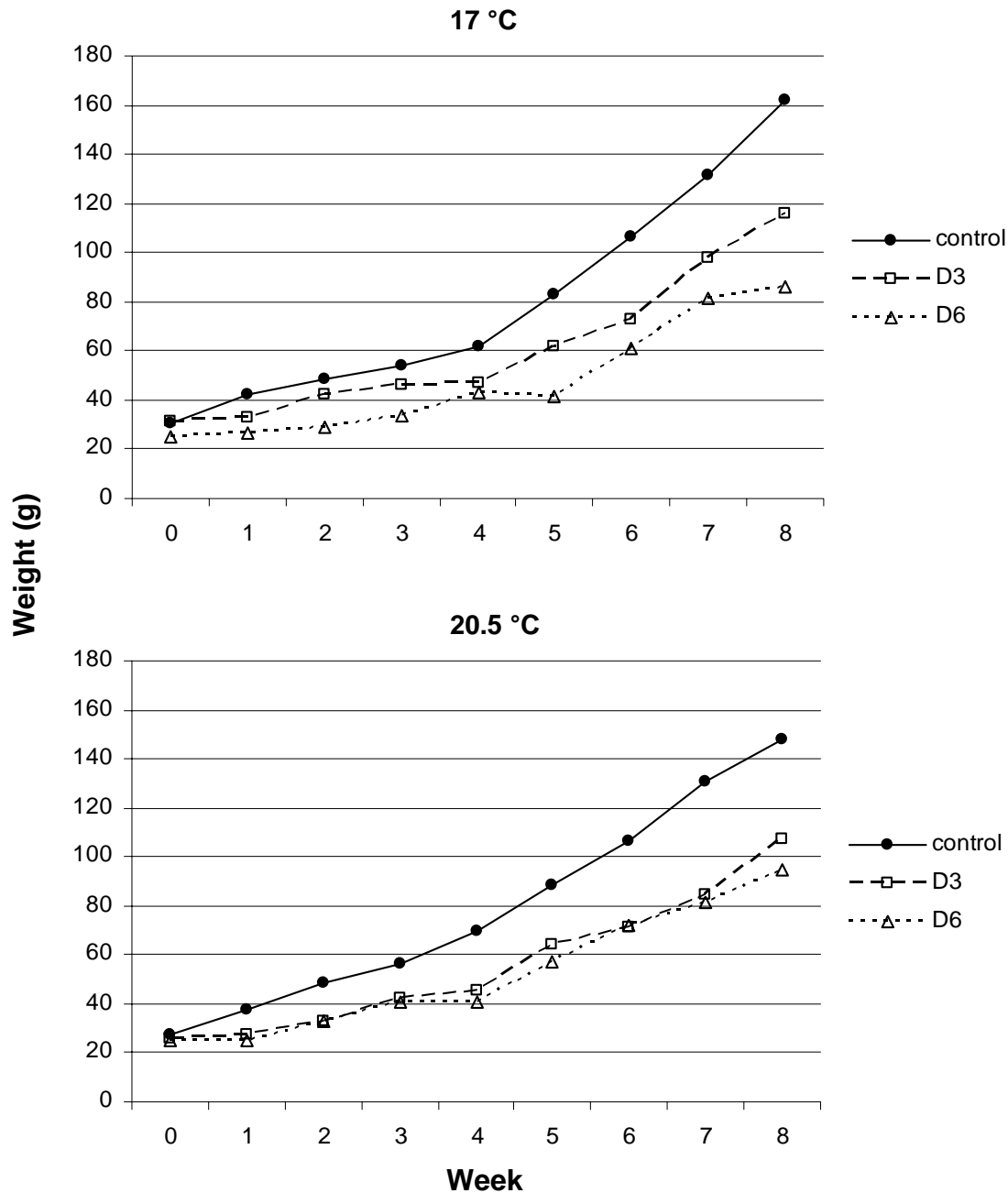
Fig. 1. Weight of 0+ rainbow trout, *O. mykiss*, measured once a week during the 8-week feeding experiment at 17 °C and 20.5 °C. Control fish were fed *ad libitum*, and fish in D3 and D6 groups were subjected to cycles of food deprivation and refeeding. Error bars have been left out for clarity.

4.2. Specific growth rate

Specific growth rate over the whole experiment of the constantly fed control fish at 17 °C was significantly higher when compared to the fish that were fasted for three (P=0.032) or six (P=0.023) days (Table 1). There were no significant differences in SGR between the two starvation groups (P=0.959) in this temperature treatment, or between any of the three feeding groups at 20.5 °C (P=0.466), even though the controls seemed to have the highest SGRs also at this temperature. Temperature had no effect on SGR (P=0.788).

However, when SGR was calculated for the time periods when the starvation groups were fed, it was significantly higher among the previously starved fish compared to the controls in four out of nine feeding periods (Fig. 2). During the remaining five feeding periods there were no significant differences between the fasted fish and the control group. It has to be noted that when comparing SGR of controls and D3 fish at both temperatures, the latter had been fed one day less during one feeding period (noted in Fig. 2), which causes their specific growth rate during the feeding period to be slightly underestimated.
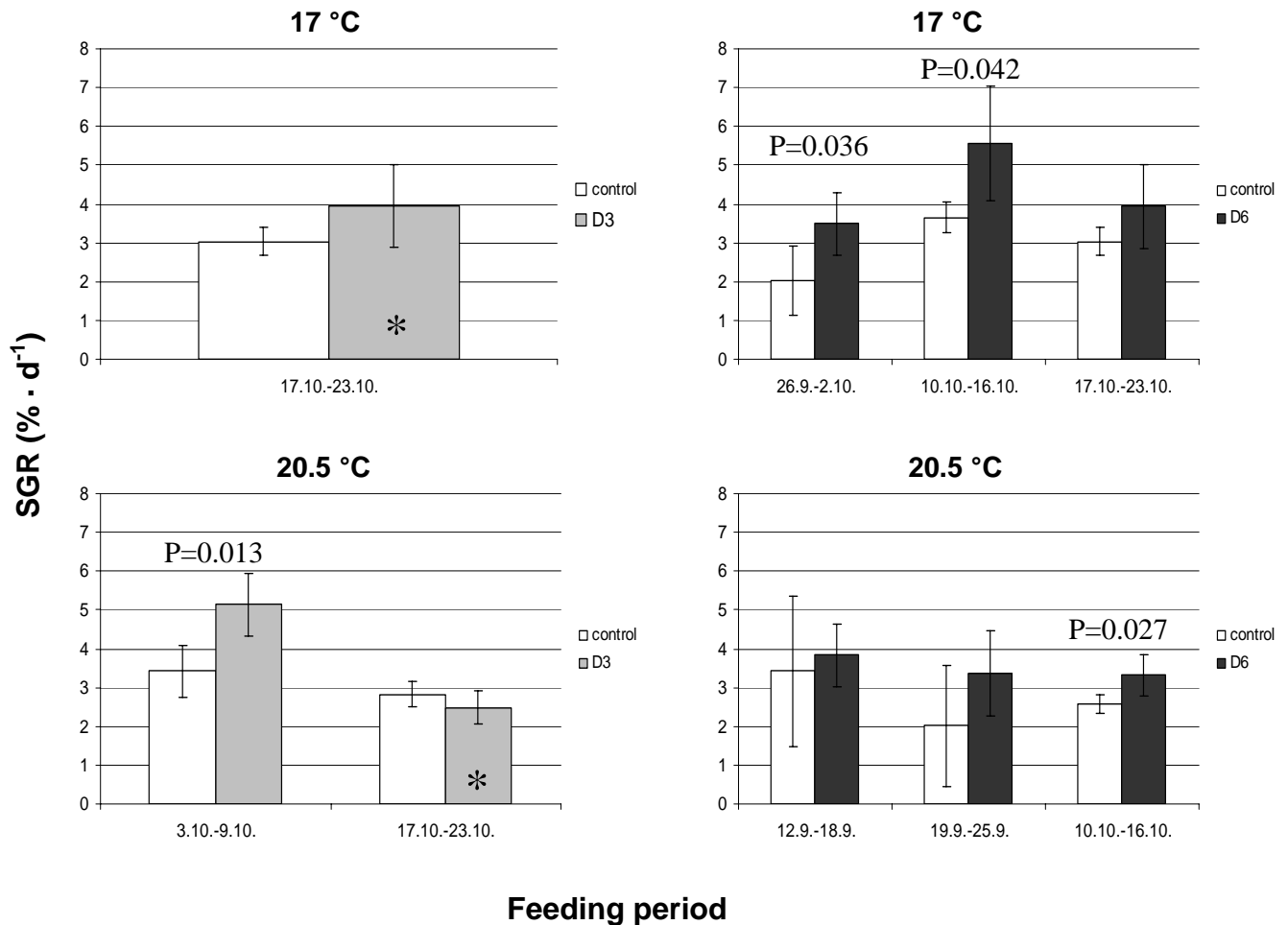

Fig. 2. Specific growth rate (SGR) of 0+ rainbow trout, *O. mykiss*, at 17 °C and 20.5 °C, during certain periods when also the intermittently starved groups D3 and D6 were fed. Bars represent group averages ± S.D. P-value denotes a statistical difference. SGRs could not be calculated for every feeding period because the feeding periods were mostly out of phase with the weekly weighing, hence the different number of feeding periods. * Group fed one day less than the control group

4.3. Food consumption

4.3.1. Relative feed intake

The food deprived fish showed signs of hyperphagia and consumed over ten percent more food than the control groups when given food (Fig. 3). However, the average relative food intake (as percentage of body weight $\cdot$ d^{-1}) of all the feeding periods of the starved fish did not differ statistically significantly from the relative food intake of the control groups during those periods, except in one case; the fish that were fasted for three days at

20.5 °C consumed on average significantly (P=0.014) more food during feeding when compared to the control fish.
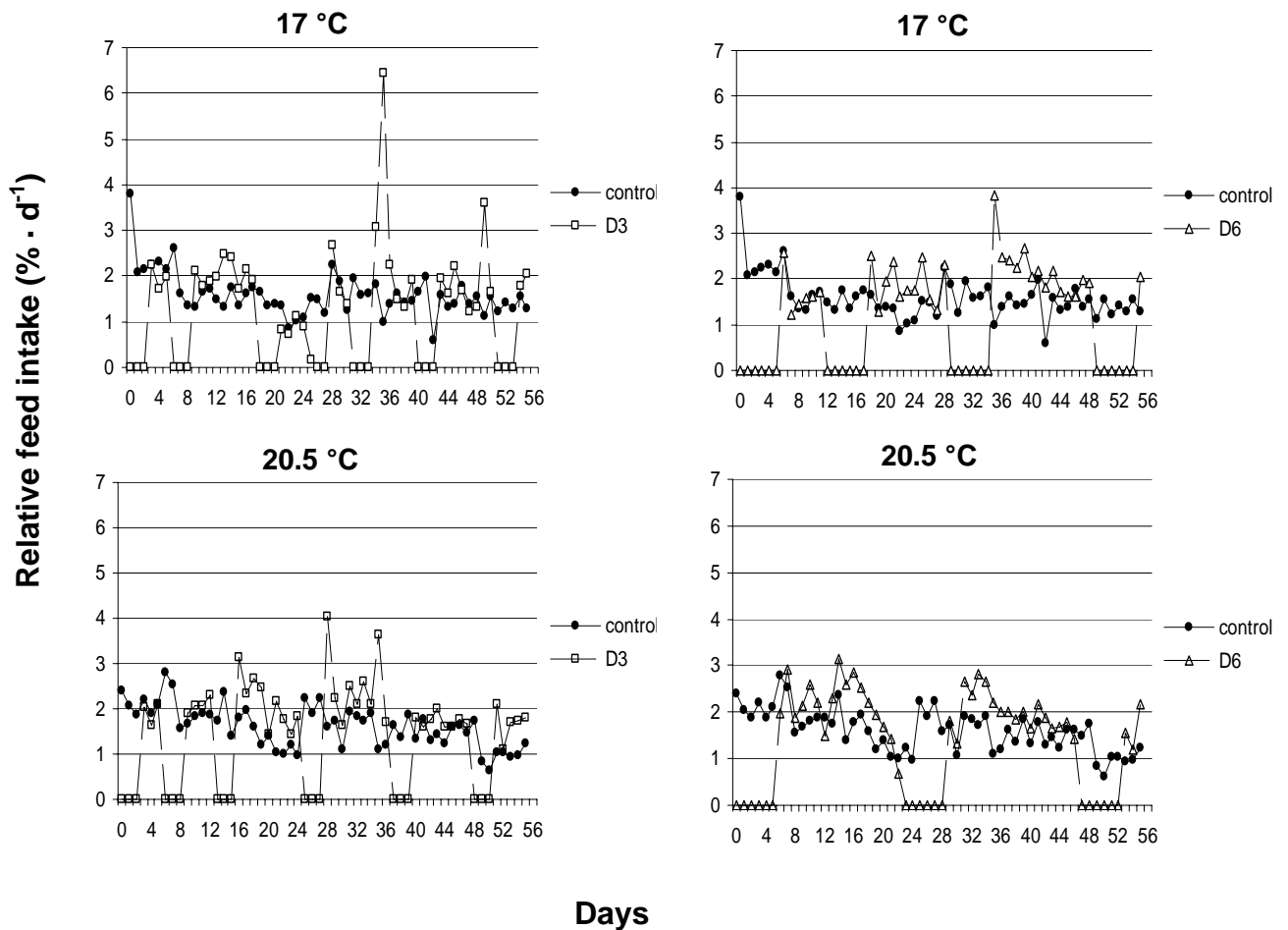

Fig. 3. Daily relative feed intake (given as percentage of body weight) of 0+ rainbow trout, *O. mykiss*, at 17 °C and 20.5 °, during the 8-week feeding experiment. Control fish were fed *ad libitum*, and fish in D3 and D6 groups were subjected to cycles of food deprivation and refeeding. Error bars have been left out for clarity.

4.3.2. Total food consumption

During the whole experiment the control fish at 17 °C consumed on average 36.6% (P=0.033) and 54.4% (P=0.003) more food than D3 and D6 fish, respectively (Fig. 4). At 20.5 °C, the corresponding percentages were 36.9% and 42.2%, but neither of these differences were statistically significant (P=0.073). Temperature had no effect on food consumption (P=0.615). A strong positive correlation was found between the weight gain of the fish and the amount of food consumed during the experiment (R^2=0.917, ANOVA P<0.001, Fig. 5). Starvation-refeeding schedule of three days led to 37.5% and 32.1% fewer days of feeding than of the continuously fed control group at 17 °C and 20 °C, respectively, and the fish that were fasted for six days had 42.9% and 32.1% less feeding days compared to the control group at 17 °C and 20.5 °C, respectively.
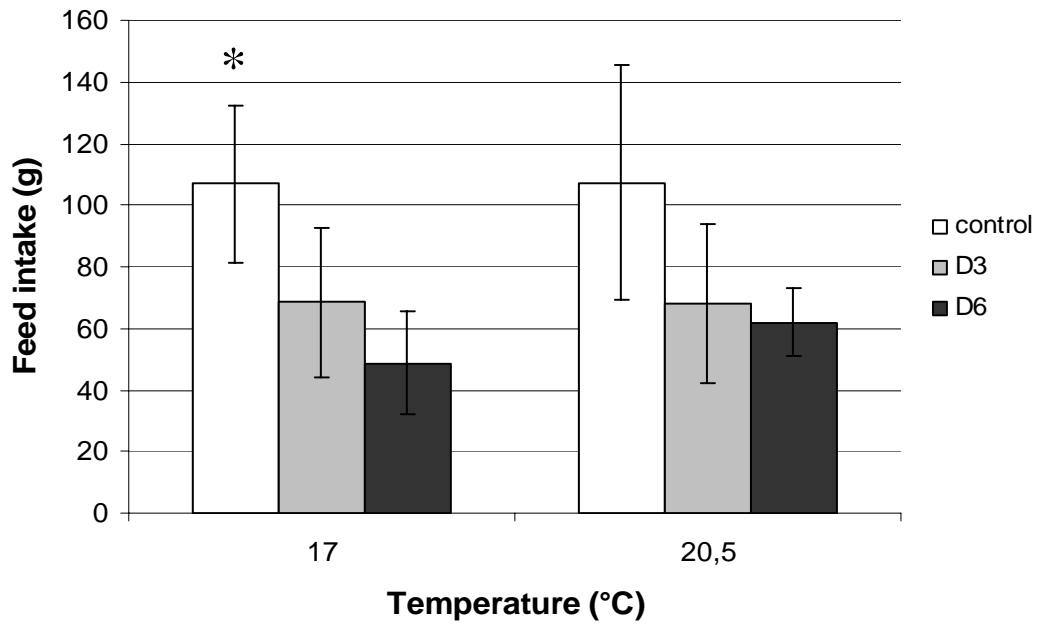
Fig. 4. Total feed intake of 0+ rainbow trout, *O. mykiss*, during the 8-week feeding experiment at two temperatures and with three feeding regimes. Bars represent group averages ± S.D. Asterisk (*) denotes a significant difference between the control group and the food deprived D3 and D6 groups at <0.05 level.
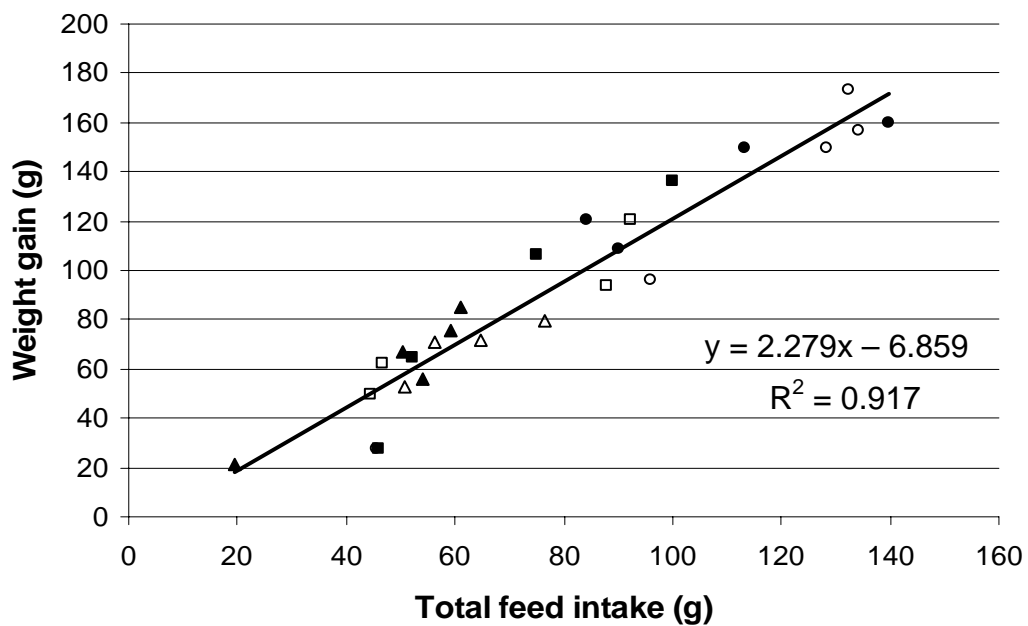

Fig. 5. Correlation of weight gain and food consumption of rainbow trout, *O. mykiss*, at the end of the 8-week feeding experiment. Filled circles, squares and triangles represent values of control-, D3- and D6-groups at 17 °C, respectively. Open circles, squares and triangles represent values of control-, D3- and D6-groups at 20.5 °C, respectively.

4.3.3. Feed efficiency

Overall feed efficiency calculated for the whole experiment did not differ significantly between different feeding regimes at 17 °C (P=0.362) or 20.5 °C (P=0.615,

Table 1). Temperature had no effect on overall FE (P=0.458). However, the fish at 17 °C had significantly better FE than the fish at 20.5 °C (P<0.001) on week seven, when the efficiencies were compared separately for different experimental weeks (Fig. 6). In addition, there was a significant interaction in FE between temperature and feeding regime on weeks three (P=0.031) and seven (P=0.024). The food-deprived groups had better efficiencies than control group at 17 °C during week seven, but at 20.5 °C the FE value of the control group exceeded those of the intermittently starved fish (differences not significant). When the two temperatures were considered separately (Fig. 6), there was a significant difference in FE at 17 °C between D3- and D6 -groups on week six (P=0.016) and on week seven (P=0.009), and between controls and D3 –fish on week seven (P=0.004). There were no significant differences in weekly FE –values between feeding groups at 20.5 °C.
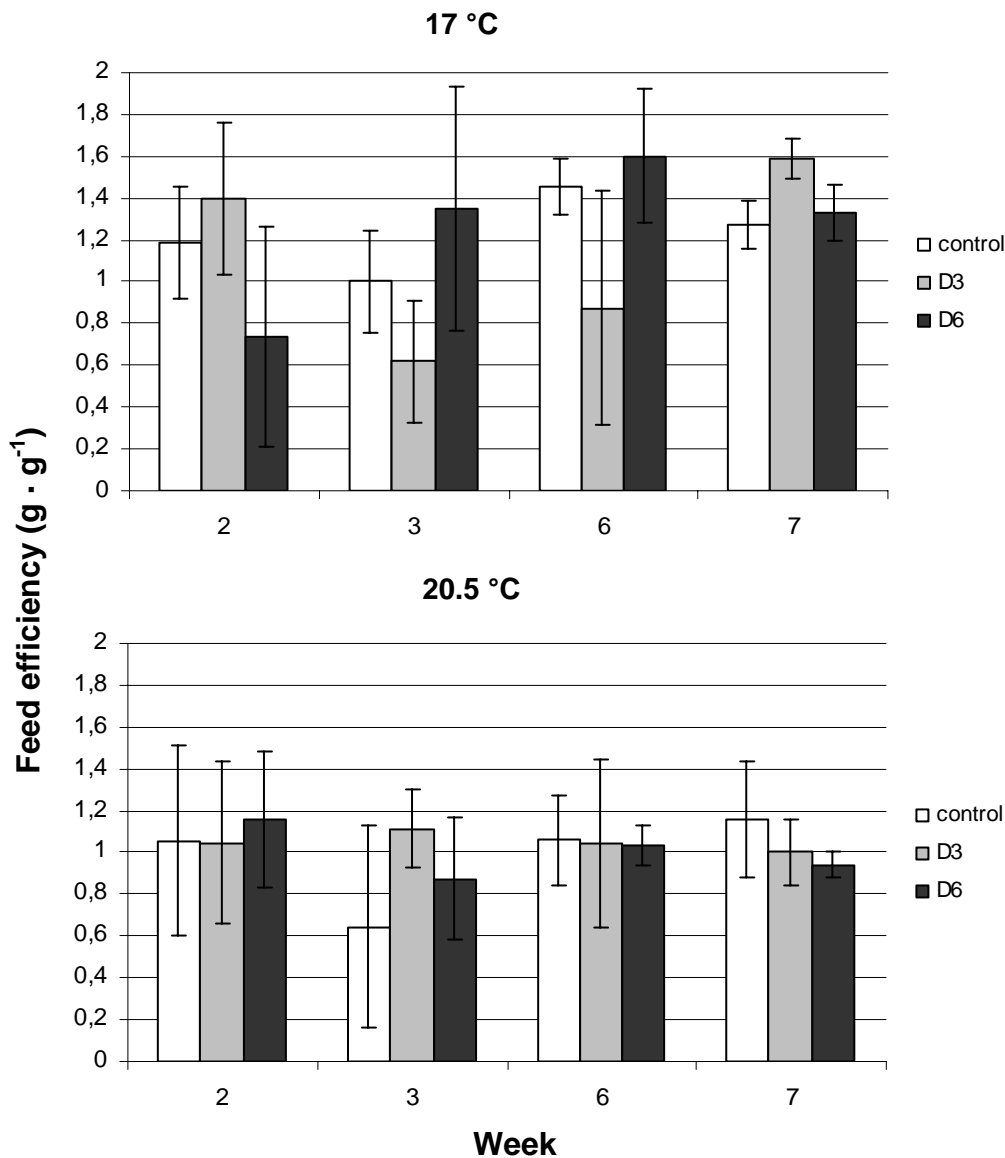

Fig. 6. Feed efficiency of 0+ rainbow trout, *O. mykiss*, during experimental weeks 2, 3, 6 and 7, at 17 °C and 20.5 °C. Bars represent group averages ± S.D.

4.4. Visceral fat

Visceral fat percentage of body weight was higher among the control group at 17 °C compared to the D3 –group (P=0.046), but did not differ from the D6 –group (P=0.559,

Table 1). At 20.5 °C, the control group had significantly more visceral fat than either of the fasting groups D3 (P=0.033) or D6 (P=0.004). There was no difference in the amount of visceral fat between the two fasting groups at 17 °C (P=0.103) or at 20.5 °C (P=0.212). Also the temperature had an effect on visceral fat percentage, which was significantly higher among the fish at 20.5 °C than at 17 °C (P=0.013). Weight of visceral fat tissue (log-transformed) correlated positively with the final weight of the fish (R^2=0.814, ANOVA, P<0.001, Fig. 7).
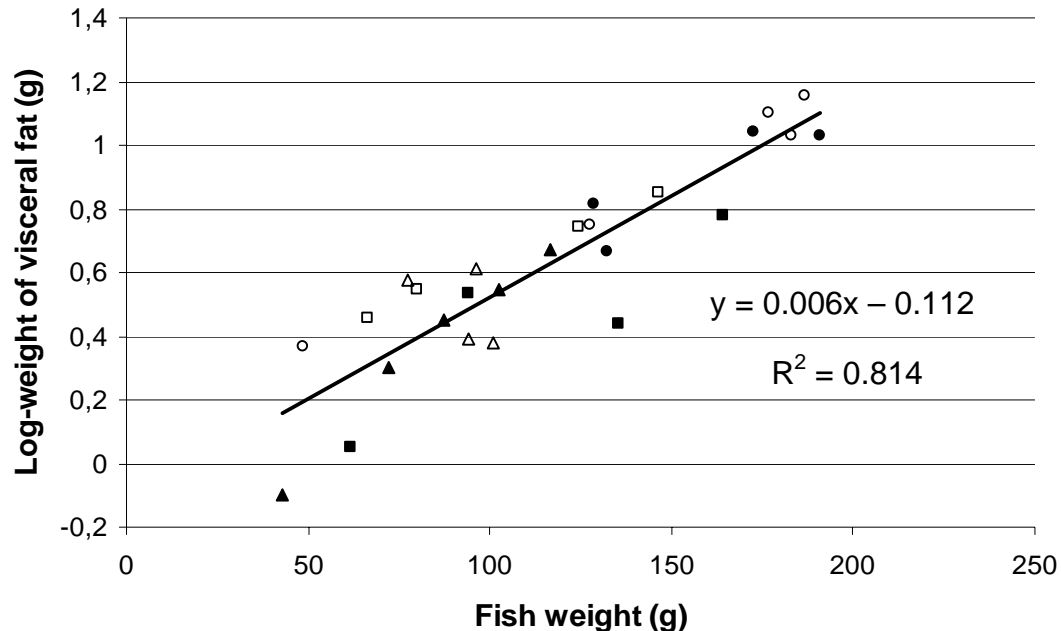

Fig. 7. Correlation of body weight and (log-transformed) weight of visceral fat tissue of rainbow trout, *O. mykiss*, at the end of the 8-week feeding experiment. Filled circles, squares and triangles represent values of control-, D3- and D6-groups at 17 °C, respectively. Open circles, squares and triangles represent values of control-, D3- and D6-groups at 20.5 °C, respectively.

4.5. Other measured morphological parameters

Dry weight of stomach content (estimate of stomach volume), stomach weight (as percentage of body weight), liver moisture or condition factor (K) did not differ significantly between the fish held at different temperatures or feeding groups (Table 1), although the controls seemed to have the highest liver moisture content at both temperatures. Hepatosomatic index (HI) was significantly lower among the control fish at 20.5 °C compared to the fish that had been intermittently starved for three days (P=0.013), but did not differ from the D6 –group (P=0.132, Table 1). Controls had the lowest HI also at 17 °C, but the differences between the different feeding groups were not significant (P=0.534). Temperature did not have an effect on HI (P=0.358). Liver wet weight correlated positively with the fish final weight (R^2=0.874, ANOVA P<0.001, Fig. 8).

4.6. Temperature

Temperature varied to some extent during the experiment (Fig. 9). The average optimum temperature was 16.8 °C, but some problems occurred with the water mixers probably due to changes in water pressure. The minimum temperature measured was as low as 12.8 °C (remained at that level for a period of three days) and peaked at 19.7 °C (one measurement). In warm water, the average temperature was 20.3 °C, but the lowest

and the highest values were measured 14.3 °C and 23.2 °C (one measurement), respectively.
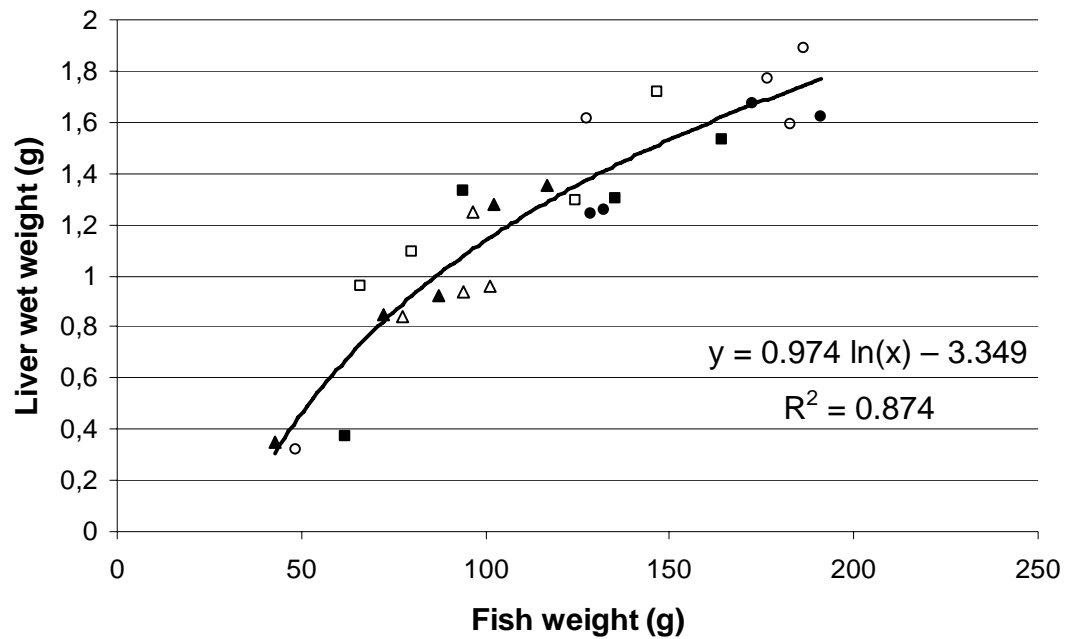
Fig. 8. Correlation of body weight and liver wet weight of rainbow trout, *O. mykiss*, at the end of the 8-week feeding experiment. Filled circles, squares and triangles represent values of control-, D3- and D6-groups at 17 °C, respectively. Open circles, squares and triangles represent values of control-, D3- and D6-groups at 20.5 °C, respectively.

Fig. 9. Water temperature at the two temperature treatments during the 8-week feeding experiment on 0+ rainbow trout, *O. mykiss*.

5. DISCUSSION

5.1. Growth and compensation

The continuously fed control fish in both temperature treatments were heavier and had greater carcass weight and total body length at the end of the experiment compared to the food deprived fish. However, the differences in weight and length were significant only between the controls and D6 –trout and indicative between c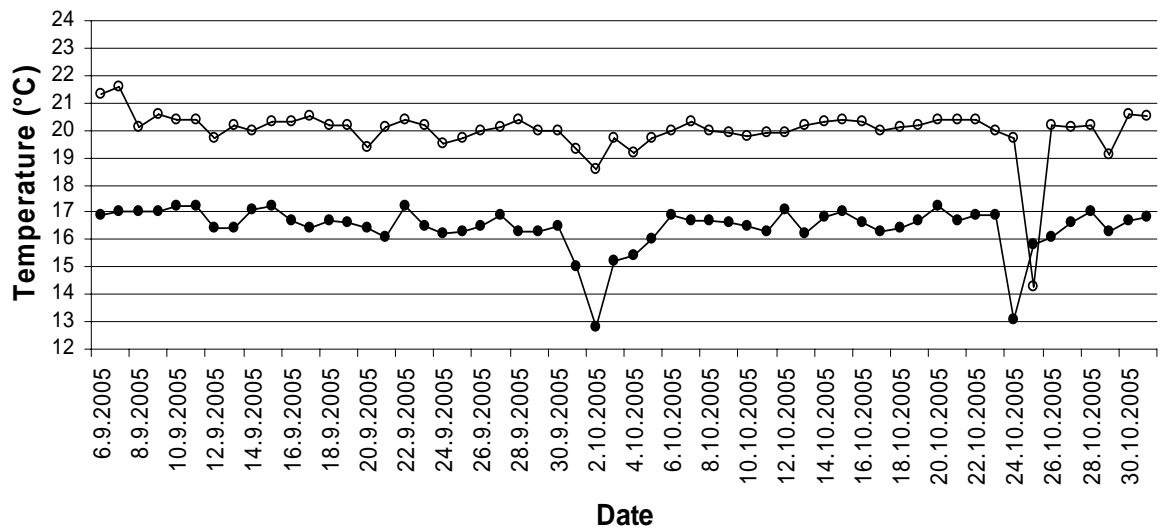ontrols and D3 -group at 17 °C, and there were no significant differences between any of the feeding groups at 20.5 °C (Table 1). The intermittently starved fish at optimum temperature could thus show only partial growth compensation. It is premature to state based on these results that the food deprived fish at 20.5 °C compensated fully in both weight and length, because the large variation especially in the values of the control fish could have masked the results at least to some extent. Increasing the number of experimental units or increasing the duration of acclimation period and thus assuring more homogenized experimental groups in terms of food consumption might have helped to reduce inter-individual variation. However, it is fairly safe to say that at least partial growth compensation occurred among all of the starved-refed rainbow trout because their growth rate (SGR) exceeded the growth rate of the control fish during the periods of feeding (Fig. 2), even though the overall growth rate remained at lower levels (Table 1). In other words, the starved-refed fish grew faster than the controls when food was available. Partial growth compensation was also observed by Miglavs & Jobling (1989b) in their experiment on Arctic charr, Wang *et al*. (2000) in hybrid tilapia and Reimers *et al*. (1993) in Atlantic salmon. Wang *et al*. (2000) suggested that hybrid tilapia had a relatively weak capacity for compensatory growth, and also keeping the fish at higher temperatures caused greater weight losses and thus prevented the fish from fully compensating. The latter conclusion is in disagreement with the results in the present study because it was in fact the warmer temperature (20.5 °C) where the rainbow trout showed better growth compensation.

Nikki *et al*. (2004) found in their experiment on rainbow trout on a similar feeding schedule where the duration of the feeding period was determined by duration of hyperphagic response, that the fish fasted for two or four days could fully compensate for lost growth and their end weight did not differ from the control values. In the present study, the lack of full compensation of fish fasted for six days at optimum temperature might be due to the relatively long fasting period where critical mass loss might have occurred preventing the fish from compensating fully. Tian & Qin (2003, 2004) suggested that only moderate feed restriction would lead to full compensatory response in barramundi. Other reason for the lack of full compensation could be the fact that the fish might have still been getting used to the intermittent feeding regime, and in order to gain full compensation the cycles of deprivation and refeeding should have been repeated for a longer period of time. The fact that the rainbow trout fasted only for three days experienced more feeding cycles and were able to compensate fully supports the previous hypothesis.

Hayward *et al*. (1997) used similar feeding regime in their experiment on juvenile hybrid sunfish and observed that the fish fasted for two and 14 days outgrew the continuously fed control fish. To my knowledge, that is the only experiment where actual growth overcompensation has been observed. Hayward & Wang (2001) hypothesized that overcompensation is species specific or limited to certain life stages or that the sunfish hybrids somehow lacked a growth limiting mechanism.

The fact that temperature had no effect on the final weight or length of the fish or on their growth rate (SGR) implies that there was no severe cost of living in the warmer

environment and that temperature did not affect on the magnitude of compensatory response. Also Dockray *et al*. (1998) found similar metabolic rates among rainbow trout living in normal and elevated (+2 °C) summer temperatures. The fact that there were no differences in fish growth between the different temperature treatments suggests a wider optimal temperature range for rainbow trout than what Jobling (1981) has proposed. However, the preferred temperature of juvenile rainbow trout is a few degrees warmer than the one of the adult fish (Jobling 1994), so probably the life stage of the fish has an effect on the temperature optimum as well. The fact that fish are able to acclimatize to increases in temperature quite well (Jobling 1994) and fluctuations of temperature in the present study (Fig. 9) might have also affected the results to some extent.

van Dijk *et al*. (2002), on the other hand, found in their study on roach that the fish starved for three weeks selected cooler water during the night, but returned to warmer water during daytime. This migration pattern was abandoned gradually upon refeeding. The control fish fed continuously selected a final temperature preference of about 27 °C which lies in the range of optimal temperature for feeding, growth and conversion efficiency of the species (van Dijk *et al*. 2002). All the starved groups showed growth compensation, even below 12 °C where the control fish did not grow at all, which suggests that after a period of food deprivation the critical temperature for growth shifts to lower levels (van Dijk *et al*. 2002). The growth compensation of the starved roach was most effective at 20 °C, which also supports the fact that food limitation causes the temperature optimum and preferendum to shift to lower levels. Also Russell *et al*. (1996) found that when given restricted rations, the growth rate of bass decreased with increasing temperature (6-18 °C). Based on the previous findings, it would be interesting to know whether the rainbow trout show better growth and stronger compensatory response when deprived of food at sub-optimal temperatures.

5.2. Food consumption

The fish that were starved for three days between feeding periods seemed to consume food in a slightly different manner than the fish that were fasted for six days between feedings. The food consumption of D3 –fish peaked more intensely at the beginning of a feeding period, but the hyperphagic response showed by the D6 –fish seemed to be less intense and continue for a prolonged period of time (Fig. 3). Russell & Wootton (1992) suggested that the compensatory response reflects the length of the prior starvation. Supporting evidence was presented also by Bull & Metcalfe (1997), and Ali *et al*. (2001) when they found that the extent of energy deficit due to increasing length of deprivation increased the duration of hyperphagia rather than the intensity of feeding.

The sudden drop in ambient temperature in both temperature treatments near the end of the experiment (Fig. 9) possibly affected the results because there is a drop also in the appetite (relative feed intake) of the control fish at 20.5 °C on day 48 (Fig. 3).

The hyperphagic response of the hybrid sunfish (Hayward *et al*. 1997) seemed to be more intense and last longer than in the present experiment on rainbow trout. In fact, when comparing the relative feed intake during feeding throughout the whole experiment, only one deprived group showed true hyperphagia; the fish deprived of food for three days at 20.5 °C consumed significantly more food than the control fish at that temperature. As Nikki *et al*. (2004) also suggested, perhaps the fish would have needed more time to get used to the intermittent feeding regime in order for more intense hyperphagic response to develop. Ali & Wootton (2000) found in their experiment on juvenile three-spined stickleback that "previous history of short-term deprivations was a factor in determining the degree of hyperphagia shown after a given period of food deprivation." Especially

when the fish were held on 1-1 days of feeding-deprivation cycle, they showed lower food consumption during the first cycle than during second or third cycle. Similar adaptation to the feeding-deprivation regime was found by Wu *et al.* (2002) when the increase in food consumption by four fish species was significant only during second, third and fourth feeding period of the feeding-deprivation cycles, and by Ali & Wootton (2001) on juvenile three-spined sticklebacks subjected to two, four or six days of starvation followed by two days of refeeding. Ali & Wootton (2001) suggested that changes in gut capacity of the starved-refed sticklebacks might have been a factor in this adaptation.

Fish consumed on average the same amount of food during feeding irrespective of the temperature treatment, thus the warmer ambient temperature did not have any deleterious effects on the appetite of the fish. Food consumption is said to increase with increasing temperature up to point where the temperature starts to approach the upper thermal tolerance limits of the organism (Jobling 1994). Dockray *et al.* (1996) found that increase in fluctuating average summer temperature depressed the appetite of rainbow trout. In the present experiment with relatively narrow temperature range, the appetite remained unaffected.

When measured as the total amount of food consumed during the whole duration of the experiment, the starved fish consumed remarkably less food than the control fish (Fig. 4) with considerably fewer feeding days. The differences in total food consumption explained most of the variation in weight gain, as can be seen from Fig. 5. Jobling *et al.* (1993) compared the weights of Arctic charr after equal number of feeding days, and found that the fish on deprivation-refeeding cycles were significantly heavier compared to the continuously fed fish. It would be interesting to know if similar results had been obtained by applying equal number of feeding days on fasted groups after cycles of deprivation and refeeding.

5.3. Feed efficiency

There was no clear effect of temperature or feeding regime on feed efficiency (Table 1, Fig. 6), possibly due to great variation between individuals. However, on the seventh week of the experiment the efficiency was significantly higher at 17 °C than at 20.5 °C, and there seemed to be a trend (although a weak one) towards the efficiency at 17 ºC to be higher than at 20.5 °C and improve as the experiment progressed (Fig. 6). Silverstein *et al.* (2000, channel catfish), Fechhelm *et al.* (1993, Arctic cisco) and Dockray *et al.* (1996, rainbow trout) found that efficiency improved when the fish were held at temperatures closer to their optimum temperature range as opposed to colder or warmer water.

Food deprivation has resulted in improved post-deprivation feed efficiency in several studies, e.g. Quinton & Blake (1990, rainbow trout), Reimers *et al.* (1993, Atlantic salmon), Boujard *et al.* (2000, rainbow trout), Qian *et al.* (2000, gibel carp) and Bélanger *et al.* (2002, Atlantic cod). Also Miglavs & Jobling (1989a) found in their study on Arctic charr that the fish were compensating by the means of better food conversion efficiency, but this lasted only for a short period of time after resuming satiation feeding, and hyperphagia was still the prevailing mechanism of compensatory growth. Gaylord & Gatlin (2000) found similar results on channel catfish, when the increase in feed efficiency among the starved fish lasted only for the first two weeks of refeeding and did not influence the overall efficiency. A long-term hyperphagic response was observed to continue still at the completion of the trial (Gaylord & Gatlin 2000). Zhu *et al.* (2001) found that minnow and three-spined stickleback were hyperphagic during compensatory phase, but improved growth efficiency was observed only in the groups starved for longer time (two weeks). It is possible that even the deprivation of six days used in the present

study was not long enough to induce improved feed efficiency. Also Hayward *et al.* (1997) observed both hyperphagia and to some extent also improved gross growth efficiency during refeeding in food deprived hybrid sunfish. Ali & Wootton (2001), on the other hand, found that growth efficiency of juvenile sticklebacks did not differ between constantly fed controls and starved-refed fish. Johansen *et al.* (2001) found poorer growth efficiency in the Atlantic salmon totally deprived of food compared to food restricted fish during the first three weeks after introducing excess feeding. The authors suggested that this might indicate an impaired digestive capacity in the deprived fish during the beginning of compensatory period.

5.4. Morphological parameters

5.4.1. Visceral fat

In most cases in the present study, the visceral fat percentage was higher among the control fish than the food deprived groups (Table 1). Nikki *et al.* (2004), however, found no differences in visceral fat percentage of rainbow trout between different feeding regimes. Weatherley & Gill (1981) found that visceral adipose tissue of rainbow trout was lost completely among the fish starved for three or 13 weeks. Also according to Quinton & Blake (1990), the proximate analysis of rainbow trout showed significant reduction in fat and increases in protein and water after three weeks of starvation. Johansen *et al.* (2001) found a trend towards reduced fat deposition in the feed-restricted Atlantic salmon, instead the restricted fish were investing more into lean body mass and dry weight during the compensatory period. According to the authors, the degree of reduction in energy (particularly fat) stores seemed to correlate with both the magnitude and duration of hyperphagic and growth responses after introduction of *ad libitum* feeding, so that the fish in the leaner group were showing stronger hyperphagic responses, and also had higher growth rates. In cod (*Gadus morhua*), only after repleting the tissues of swimming muscles the fish start to deposit energy rich lipid reserves in the liver (Jobling 1994). The findings in the previous studies support the theory that fat (especially visceral fat tissue) is very labile and readily utilized during a period of food deprivation or restriction and is replaced last when adequate feeding is restored. One explanation for the difference between the results in the present experiment and the study by Nikki *et al.* (2004) could be the fact that the rainbow trout in the present study might have not yet been able to replete their fat reserves by the time of sampling, and were still investing more into lean body mass.

Lipid reserves in rainbow trout increased with increasing body weight in an experiment by Weatherley & Gill (1983). Also in the present study the wet weight of visceral fat correlated positively with the fish weight (Fig. 7).

It is impossible to draw conclusions whether the results of the present experiment support the lipostat model (Jobling & Johanssen 1999) or not, because only the amount of visceral fat, not whole body fat, was measured. In addition, the amount of visceral fat was only determined at the termination of the experiment, thus the lipid status of the fish in relation to the compensatory response (periods of hyperphagia) is impossible to determine.

Also the temperature had an effect on the visceral percentage of body weight. The amount of visceral adipose tissue was significantly higher among the rainbow trout at 20.5 °C than the fish held at 17 °C. One possible explanation for this might be the fact that perhaps lipid metabolism is faster or more efficient at warmer surroundings. Also Weatherley & Gill (1983) found that at low temperature (7 °C) the satiation fed rainbow trout had lower lipid and higher protein content than the fish held at 12 °C. Russell *et al.*

(1996), however, found opposite results when the visceral percentage of juvenile sea bass decreased with increasing temperature (6-18 °C) with significant negative correlation.

5.4.2. Stomach and liver

Even though the size of stomach (weight, estimate of volume) was larger among the fish intermittently starved for three days at 17 °C and relatively large also at 20.5 °C, the differences were not significant compared to the other groups (Table 1). Also Nikki *et al.* (2004) found that feeding regime did not have significant effect on the stomach volume of rainbow trout. Weatherley & Gill (1981), on the other hand, discovered that the gut of rainbow trout starved for 13 weeks reduced in size, but after a recovery phase the gut weight of the fish was significantly larger than that of the control fish. Also Simpkins *et al.* (2003) found the gut somatic index of rainbow trout declining linearly over 147 days of food deprivation and that the changes were more pronounced among active fish. Bélanger *et al.* (2002) starved Atlantic cod for ten weeks and found that after refeeding the relative mass of pyloric caeca (involved in synthesis and secretion of digestive enzymes and nutrient absorption) was 1.2 times higher in the deprived group, but the difference was not significant. Of the enzymes involved in digestion, the activity of citrate synthase was significantly higher among the food deprived fish. Also other digestive enzyme activities were higher among the deprived fish, but the differences were not significant. Intestine somatic index was 1.2 times higher in the deprived group (but not significant).

Hepatosomatic index was lowest among the control fish at both temperatures (significant only in one case, Table 1), which is probably just a consequence of a greater body size. As the size of the fish increases, the proportion of liver mass decreases in relation to body mass. It can also be seen from Fig. 8 that the liver weight does not correlate linearly with fish weight, but the increase in liver mass slows down when greater body weight is reached. Also Weatherley & Gill (1981) obtained similar results in their experiment on rainbow trout when they found that after a recovery phase the liver wet weight was larger among the starved fish than among the controls. Nikki *et al.* (2004), on the other hand, found no difference in liver weight between the food deprived and continuously fed rainbow trout. Gaylord & Gatlin (2000) found that hepatosomatic index of channel catfish was the most responsive index to food deprivation and realimentation. During deprivation, liver reduced in mass dramatically, but the composition stayed approximately the same except for the glycogen concentration (Gaylord & Gatlin 2000). The authors hypothesized that liver glycogen stores may be depleted initially in response to deprivation but are repleted during prolonged fasting by gluconeogenesis. Liver lipid was elevated during the period of deprivation (Gaylord & Gatlin 2000). Simpkins *et al.* (2003), however, found that hepatosomatic index of rainbow trout remained relatively unchanged for 105 days of food deprivation, supporting the fact that liver function was somehow defended. The authors came to the conclusion that other sources of lipid and protein were depleted before liver tissues. When the amount of body lipid decreased to levels less than 3.2 %, liver function was compromised, and the fish died (Simpkins *et al.* 2003).

6. CONCLUSIONS

Rainbow trout is the most important cultured fish species in Finland and widely farmed fish also throughout the world. The production of rainbow trout in Finland peaked in 1991 with 19.1 million kg of cultured fish (Seppälä *et al.* 2001). After that the production has declined gradually and in 1999 rainbow trout was cultured approximately 15.3 million kg (Seppälä *et al.* 2001).

Increasing productivity with as low costs as possible has always been one of the main interests in fish farming. Especially improving feed efficiency (better growth with less feed) and the ways in which it could be applied to fish farming has been one of the main goals in studies focusing on fish growth. In many studies involving compensatory growth the starved-refed fish have been able to grow as well or even better than their continuously fed conspecifics through increased efficiency or hyperphagia, or in some cases, both. In other words, the fish have grown as well as the controls but with fewer resources. By applying food deprivation-refeeding cycles in fish farms it could be possible to create more flexible feeding regime, and also less manpower would be needed with fewer feeding events, especially in farms where the fish are fed by hand. In addition, Kindschi (1988) found that when rainbow trout were starved and fed on a four-week interval, the variation in size was less than among controls (less aggressiveness and fighting for food). So if uniform size is desired in culture facilities, intermittent feeding could be an option. Also the quality of fish as a food source could be improved by intermittent feeding regime. In the present study, the amount of visceral fat tissue was lower in rainbow trout held at optimum temperature as opposed to warmer water, and in starved fish compared to the continuously fed controls. Thus the proportion of edible flesh could be maximized by using periods of fasting and refeeding and appropriate temperature range. On the other hand, taking cycles of deprivation and refeeding in to use instead of continuous feeding, it could take more time until the fish would reach adequate size for markets, which could in turn lead to decreased productivity. In addition, finding such feeding-deprivation regime where the fasting period is long enough to evoke intense hyperphagic response and perhaps even increased feed efficiency, without being too severe and leading into incomplete growth compensation, is difficult. Further studies are needed in order to find optimal conditions for full compensatory response or overcompensation in rainbow trout culture.

Leaching of nitrogen and phosphorous into aquatic environment causes eutrofication, which can alter the sensitive ecosystems in the Baltic Sea and the shallow lakes that are typical in the Finnish landscape. In 1999, fish farms were responsible for 994 tons of nitrogen and 121 tons of phosphorous discharges into the environment in Finland, and in some Finnish coastal areas, fish farming was the most important source of nutrients (Seppälä *et al*. 2001). By finding optimal conditions in rainbow trout culture via increased feed efficiency and reduced levels of feed waste (increased appetite could be reached through fasting), the amount of nutrients leaching from fish farms into the environment could be remarkably reduced.

The effect of temperature and food deprivation together on fish growth and well being has not yet been studied well enough, and it certainly deserves more attention in the future, especially when the average temperature of water bodies is gradually rising due to global warming. Future research should also concentrate on the phenomenon of compensatory growth in natural conditions, and more manipulative experiments on field are needed.

ACKNOWLEDGEMENTS

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Appendix. The experimental settings in the 8-week feeding experiment on rainbow trout, *O. mykiss*.