Vurdering af ernæringstilstand for aborre

af

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Vurdering af ernæringstilstand hos opdrættede aborrer

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INTRODUKTION

I ethvert dyrehold er det vigtigt at kunne vurdere dyrenes ernæringstilstand for at sikre dyrenes velfærd og opnå en optimal udnyttelse af det tildelte foder. I forbindelse med aborreprojektet: "Udvikling af opdræt af aborre – en mulig alternativ art i ferskvandsopdræt" blev der observeret tilfælde af pludselige dødsfald blandt de største og tilsyneladende sundeste fisk. En nærmere undersøgelse viste at disse fisk havde kraftige aflejringer af fedt omkring organerne i bughulen. Der er derfor meget der tyder på at netop disse store, dominante individer er døde af et metabolisk stress på grund af meget stort fødeindtag. Med andre ord, at de havde ædt sig ihjel. En reduktion af fodertildelingen betød at disse problemer ophørte. Det blev derfor besluttet at undersøge hvilke måleparametre, der kan anvendes til vurdering af ernæringstilstand.

MATERIALE OG METODER

130 stk. 11 måneder gamle aborrer (gennemsnitsvægt 200g) blev udtaget tilfældigt blandt de opdrættede aborrer klækket i maj 2003, d.v.s. ca. et år gamle. Fiskene blev anbragt i to 7 m³ kar og holdt ved 20 °C fra 30 marts 2004 til 26. juli 2004. Vandet blev recirkuleret med mindre end 3% vandudskiftning pr. dag. Den ene gruppe blev fodret normalt, mens den anden gruppe blev sultet. Den fodrede gruppe blev fodret med 2mm Dan-Ex 1344 i en mængde svarende til 1,5% af kropsvægten hver anden dag. Dan-Ex 1344 indeholder 13% lipid, 44% protein, 25% kulhydrat og har et energiindhold på 4826 Kcal/kg. Den sultede gruppe blev sultet fra forsøgsstart til 29 juni 2004 hvorefter den blev fodret som den fodrede gruppe indtil forsøgsafslutning 26. juli 2004.

Hver 14 dag blev tilfældigt udtaget fem fisk fra hver gruppe. Fiskene blev nedfrosset for senere analyse.

Efter forsøgets afslutning blev følgende måleprogram gennemført:

- 1. Totallængde måles til nærmeste mm nedad.
- 2 Vådvægt registreres efter let aftørring nøjagtighed 0.1g
- 3. Bughulen åbnes og indvoldene udtages. Leveren fjernes
- 4. Leveren vejes.
- 5. Til tørvægt afskæres ca. 1/3 der vejes med max. nøjagtighed.
- 6. Tørvægtsstykket anbringes i varmeskab 58°C,i 24 timer. Vejes derefter med max. nøjagtighed
- 7. Indvolde vejes efter at mavesæk er åbnet og maveindhold fjernet.
- 8. Fileter afskæres og skindet fjernes. Fileter vejes (0.1g)

9. Til tørvægt afskæres en strimmel på ca. 1 cm. midt på fileten, vejes med max. Nøjagtighed

10. Tørvægtsstykket anbringes i varmeskab 58deg 24h. Vejes derefter med max. nøjagtighed

Prøver for fem fisk samles og der registreres

- 1. Samlet vådvægt
- 2. Samlet mesenterievægt
- 3. Samlet levervægt.

RESULTATER OG DISKUSSION:

Rådata for forsøget er præsenteret i tabel I.

Ved hver prøvetagning blev udtaget fem fisk pr. gruppe. Da fiskene ved indgang i forsøget varierede i længde fra ca. 19 til 25 cm., og i vægt fra ca. 100 til 250 gram medfører størrelsesvariationen relativt stor variation indenfor den enkelte prøvetagning og mellem prøver. Da formålet med undersøgelsen var at finde praktisk anvendelige mål for ernæringstilstande blev det vurderet at for at en parameter skal være egnet til praktisk brug må der kunne ses en effekt ved måling på fem fisk.

Total længde (figur 1.)

Der kunne observeres en stigning i gennemsnitlig total længde i forsøgsperioden for de fodrede fisk, mens gennemsnitslængden for de sultede forblev relativt konstant. Total længde vil kun kunne anvendes som udtryk for ernæringstilstand ved at sammenholde med andre grupper af fisk holdt ved samme temperatur.

Våd vægt (figur 2.)

Der kunne observeres en stigning i vådvægt for de fodrede fisk mens vådvægt forblev relativt konstant hos sultede fisk. Da fiskenes stofskifte medfører et forbrug af fedtstoffer og protein ville der kunne forventes et fald i vådvægt såfremt vægttabet ikke kompenseres af et øget vandindhold i fisken.

Tørvægtsprocent (figur 3.)

Vandindholdet i fileten blev målt for at afklare om et vægttab under sult kompenseres af et øget vandindhold. Resultaterne viste at tørvægtsprocenten er lavere i sultede fisk (= højere vandindhold) og faldende med længere sultperiode. Efter genoptaget fodring observeres en kraftig stigning i i tørvægtsprocent. Tørvægtsprocent kan derfor være en egnet indikator for ernæringstilstand. Forskelle i tørvægtsprocent kan i øvrigt tænkes at påvirke hvorledes fiskens kødkvalitet opleves af forbrugerne.

Kondition (Condition factor K) (figur 4.)

Fiskens kondition er et udtryk for sammenhængen mellem længde og vægt således at højere vægt ved en given længde betyder en højere kondition. Hvis fisken vokser lige meget på alle leder (isometrisk) vil konditionen være konstant idet kondition beregnes som $K = våd vægt/længde^3$. Det kan ses at konditionen er højest hos de fodrede fisk og faldende i sultperioden. Efter genoptagelse af fodring stiger konditionen igen.

Vægt af indre organer (mesenterie) (figur 5.)

Aborre aflejrer fedt omkring de indre organer som reserve næring bl.a. til brug under opbygningen af kønsprodukter. Vægten af mesenteriet kan derfor forventes at være højere hos fodrede end hos sultede fisk. Dette kan tydeligt observeres i forsøget hvor fodrede fisk har en mesenterievægt omkring 15g mens vægten hos de sultede er ca. det halve. Forskellen optræder allerede inden for den første måned efter forsøgets start. Det bemærkes at mesenterievægten ikke stiger efter genoptaget fodring. Dette skyldes formentlig af de genfodrede fisk først reetablerer normale fysiologiske forhold i kroppen førend der deponeres fedtreserver i mesenteriet.

Mesenterievægt i procent af fiskevægt (figur 6.)

Da vægten af mesenteriet er en funktion af fiskens størrelse blev det undersøgt om mesenterievægt i procent af vådvægt er et brugbart udtryk for ernæringstilstand. Resultaterne viste at

mesenterievægten udgjorde 4-5% af vådvægten hos sultede fisk og 7-8% af vådvægten hos fodrede fisk. Når mesenterievægten stiger i forhold til den totale kropsvægt kan det skyldes at kropsvægten påvirkes af den genoptagne fodring gennem reduktion af vandmængden i vævene.

I praksis vil der kunne foretages en bestemmelse af mesenteriets størrelse og fedtindhold ud fra en rent visuel inspektion af de indre organer.

Levervægt (figur7.)

Hos mange fiskearter (f.eks. torsk) er leveren opmagasineringsorgan for reservenæring. Det betyder at en fisk i god ernæringstilstand vil ophobe fedtstoffer i leveren. Derigennem bliver leveren større. Samtidigt vil leverens tørvægtsprocent falde da vandindholdet i selve levervævet kommer til at udgøre en forholdsvis mindre andel af total vægten for leveren. Målinger af levervægt viste at vægten hos fodrede fisk lå omkring 2gram og hos sultede fisk omkring 0,5g med en stigning til ca. 1g efter genoptagelse af fodring.

Levervægt som % af vådvægt (figur 8.)

Hvis levervægten beregnes som % af fiskens vådvægt bliver den observerede stigning i slutningen af forsøget endnu tydeligere, formentlig på grund af ændringene i fiskenes vandindhold efter genoptagelse af fodring.

Lever tørvægtsprocent (figur 9.)

Tørvægtsprocenten i leveren ser ud til at stige under sult, og er ikke tydeligt forskellig for fodrede og for sultede fisk. Dette indikerer at leveren hos aborre kun i begrænset omfang fungerer som energidepot.

Lipidindhold (Tabel II)

Fedtindholdet i lever, indvolde og i filet er målt med Bligh & Dyers metode. Resultaterne viste at der hverken i lever eller filet var klare effekter af sult på fedtindholdet. Derimod var der allerede et tydeligt fald i fedtindholdet i indvoldene efter 14 dages sult. Det lavere niveau kunne, med nogen variation, følges gennem hele sultperioden. Fedtindholdet i leveren er klart lavere end de niveauer der ses hos fiskearter hvor leveren fungerer som energidepot. Tabel II.

	LE	/ER	FILE	T	INDVOLDE		
Dato	Fodret	Sultet	Fodret	Sultet	Fodret	Sultet	
15-04-2004	4,77	5,3	1,29	1,2	50,83	55,03	
30-04-2004	5,42	8,1	1,51	1,37	67,76	40,47	
14-05-2004	2,62	11,84	1,09	1,05	61,12	48,58	
01-06-2004	4,42		1,29	1,33	57,63	60,37	
15-06-2004	4,17	8,13	1,23	1,05	67,2	55,27	
28-06-2004	4,63	6,91	1,44	1,35	67,31	45,09	
13-07-2004	4,56	3,95		1,03	57,57	34,15	

Tabel II. Fedtindhold i % af tørvægt for lever, filet og indvolde. Gennemsnitsværdier for fem fisk.

KONKLUSION

For de fleste af de målte parametre kunne observeres tydelige effekter af sult allerede efter to uger. Disse effekter var fortsat tydelige i de første to måneder af sultperioden. Efter genoptagelse af fodring blev sulteffekterne hurtigt mindre tydelige.

Resultaterne viste at vægten af indvolde i forhold til fiskens totalvægt er mest egnet for rutinemæssig vurdering af ernæringstilstand. Effekten af sult på mængden af fedt omkring indvoldene var så tydeligt at en visuel inspektion vil kunne give god indikation af fiskens ernæringstilstand. Parametre som leverindex (levervægt i procent af vådvægt) og levertørvægtsprocent er ikke egnede for aborre, mens kondition kan anvendes ved længere sultforløb. Kondition har desuden den fordel at den kan måles på levende fisk.

Tabel I.

			FISH	FISH	FISH	FILLET	MESENT	MESENT	LIVER	LIVER	FILLET	LIVER
Date	Group	FISK No.	TL	Wet weight	Cond	Wet weight	Wet weight	% of WW	Wet weight	% of WW	DW %	DW %
15-04-2004	control	control 8	20,1	121,91	1,501	37,642	7,504	6,155	0,601	0,493	23,03	22,15
15-04-2004	control	control 3	19,6	104,69	1,390	30,196	5,949	5,682	0,878	0,839	23,48	28,57
15-04-2004	control	control 7	20,2	112,60	1,366	31,771	6,201	5,507	0,529	0,470	23,20	26,84
15-04-2004	control	control 1	22,3	1/4,8/	1,577	55,604	12,738	7,284	1,552	0,888	23,85	29,03
15-04-2004	control	control 9	19,1	112,74	1,018	32,429	7,409	6,010	0,982	0,871	22,00	31,03
15-04-2004	starvation	starva f	21,9	200.10	1,407	47,007	9,701	0,390	1,003	0,094	23,32	21,27
15-04-2004	starvation	starva 0	23,0	200,19	1,525	26.824	7 320	7,430	0,730	0,308	20,19	20,99
15-04-2004	starvation	starva 8	21.9	163.75	1,417	49 241	15 116	9,000	0,243	0,252	22,20	20,44
15-04-2004	starvation	starva 5	19.9	103,13	1,309	28 912	4 336	4 204	0,100	0,404	20,30	21,13
30-04-2004	control	control 1	24.6	252 52	1,005	72,365	26.94	10.668	3 115	1 234	23.64	35.46
30-04-2004	control	control 2	23.5	184 11	1 4 1 9	53 934	15 328	8,325	3,000	1,201	22,85	28.37
30-04-2004	control	control 3	21.5	133.04	1.339	45.628	7.292	5,481	0.938	0.705	22.33	30.04
30-04-2004	control	control 4	23.8	216.82	1,608	65.957	19.64	9.058	2,738	1,263	22.34	29.63
30-04-2004	control	control 5	24,9	248,72	1,611	83,79	20,323	8,171	2,904	1,168	22,05	28,23
30-04-2004	starvation	starva 1	23,5	182,62	1,407	58,172	11,619	6,362	0,662	0,363	22,39	21,06
30-04-2004	starvation	starva 2	19,6	91,37	1,213	25,867	4,232	4,632	0,247	0,270	20,96	19,90
30-04-2004	starvation	starva 3	21,3	147,52	1,527	43,401	12,678	8,594	0,673	0,456	22,61	25,37
30-04-2004	starvation	starva 4	18,4	71,302	1,145	21,283	3,124	4,381	0,236	0,331	19,69	19,98
30-04-2004	starvation	starva 5	20,4	112,17	1,321	29,799	7,172	6,394	0,275	0,245	21,20	24,75
14-05-2004	control	control 2	21,5	142,45	1,433	26,317	8,91	6,255	0,540	0,379	22,74	25,71
14-05-2004	control	control 5	24,3	228,73	1,594	57,014	18,953	8,286	1,184	0,518	23,90	28,53
14-05-2004	control	control 4	22,1	143,33	1,328	31,494	8,428	5,880	1,372	0,957	24,04	23,44
14-05-2004	control	control 9	22,7	162,56	1,390	39,491	12,196	7,502	1,079	0,664	23,87	26,62
14-05-2004	control	control 10	19,1	95,58	1,372	23,673	5,779	6,046	0,575	0,602	23,84	25,17
14-05-2004	starvation	starva 1	20,5	113,73	1,320	27,509	6,675	5,869	0,190	0,167	23,74	25,04
14-05-2004	starvation	starva 9	21,0	120,13	1,297	33,147	6,762	5,629	0,452	0,376	23,36	23,24
14-05-2004	starvation	starva /	23,5	1/5,41	1,352	39,627	8,623	4,916	0,681	0,388	22,03	20,76
14-05-2004	starvation	starva 3	21,1	127,59	1,308	29,801	5,788	4,530	0,388	0,304	22,49	23,30
14-05-2004	starvation	Starva Z	24,0	100,00	1,342	42,243	9,177	4,947	0,415	0,224	22,04	24,00
01-06-2004	control	control 10	23,8	202,89	1,505	30,984	19,411	9,507	1,091	0,538	22,00	29,03
01-06-2004	control	control 2	21,2	140,90	1,032	21,472	10,040	6 1 1 6	1,400	0,959	23,00	20,00
01.06.2004	control	control 9	24,4	203.43	1,591	52 703	14,130	0,110	1,052	0,455	22,10	20,02
01.06.2004	control	control 5	22,0	201,40	1,710	46,401	17 231	8 543	2 805	1 435	22,04	26.76
01-06-2004	starvation	starva 8	19.3	85.93	1 1 1 9 5	14 228	3 894	4 532	0 160	0 186	21 45	27,10
01-06-2004	starvation	starva 6	20.6	116 75	1,336	15 702	4 732	4 053	0,100	0,100	21,40	26.48
01-06-2004	starvation	starva 4	18.5	72,565	1,146	14,195	2,437	3,358	0,153	0,211	21.84	24.16
01-06-2004	starvation	starva 5	23.5	183.79	1,416	30,856	9,439	5,136	0.387	0.211	21.38	28.49
01-06-2004	starvation	starva 2	23,6	185,68	1,413	41,324	10,544	5,679	0,401	0,216	22,07	21,89
15-06-2004	control	control 1	23,2	188,59	1,510	55,11	12,839	6,808	3,05	1,617	24,91	28,55
15-06-2004	control	control 2	24,6	247,62	1,663	62,883	17,833	7,202	2,680	1,082	24,33	25,14
15-06-2004	control	control 3	25,6	242,12	1,443	74,068	16,494	6,812	3,45	1,425	24,20	25,86
15-06-2004	control	control 4	20,5	186,11	2,160	47,135	13,126	7,053	1,232	0,662	23,89	28,40
15-06-2004	control	control 5	25,0	230,18	1,473	75,931	17,545	7,622	2,699	1,173	24,59	24,27
15-06-2004	starvation	starva 1	21,6	126,29	1,253	33,044	6,234	4,936	0,249	0,197	22,71	27,13
15-06-2004	starvation	starva 2	21,4	116,45	1,188	33,636	1,647	1,414	0,287	0,246	21,95	23,53
15-06-2004	starvation	starva 3	21,0	113,28	1,223	29,642	3,738	3,300	0,255	0,225	22,46	34,73
15-06-2004	starvation	starva 4	22,6	162,37	1,407	37,766	/,814	4,812	0,457	0,281	21,86	24,33
15-06-2004	starvation	starva 5	24,6	201,58	1,354	48,898	15,301	7,591	0,424	0,210	22,39	28,10
28-06-2004	control	control 1	25,6	256,06	1,526	44,642	20,791	8,120	2,923	1,142	23,77	22,97
28-06-2004	control	control 2	24,2	210,43	1,485	/4,663	12,963	6,160	2,114	1,005	24,44	29,87
28-06-2004	control	control 3	22,5	149,29	1,311	24,601	9,588	0,422	1,81/	1,217	23,92	26,72
20-00-2004	control	control 5	23,7	221,43	1,003	41,008	10,729	7,103	3,309	1,494	24,91	30,90
28.06.2004	staniation	stanya 1	20,0	207,05	1,000	24 890	2 214	1,100	2,304	0.257	24,04	01,10 00 10
28-06 2004	starvation	starva 2	20,2	185.02	1 212	24,009	3,214	5,312	0,249	0,237	21,70	22,13
28-06-2004	starvation	starva 3	24,2	105,93	1,312	28 373	2 800	2745	0,009	0,274	22,19	19.74
28-06-2004	starvation	starva 4	20,7	128 13	1 487	34 724	6 799	5 306	0,231	0,230	21,74	24 47
28-06-2004	starvation	starva 5	25,0	202 54	1 296	55 963	9 722	4 800	0.593	0 293	21 76	21.57
13-07-2004	control	control 1	25.6	270.6	1 613	88.06	20 124	7 437	2 448	0,905	23.22	28.86
13-07-2004	control	control 2	25,6	257.39	1,534	77.116	22.349	8.683	3.272	1.271	22,49	28.90
13-07-2004	control	control 3	23.1	188.72	1.531	66.576	10.895	5,773	1.228	0.651	22.11	25.38
13-07-2004	control	control 4	23.6	198.47	1.510	66.301	14.627	7.370	2.063	1.039	22.90	30.55
13-07-2004	control	control 5	25,1	270,15	1,708	83,41	20,029	7,414	3,091	1,144	22,22	26,45
13-07-2004	starvation	starva 1	19,1	91,23	1,309	21,298	4,046	4,435	0,873	0,957	21,31	24,51
13-07-2004	starvation	starva 2	24,1	213,4	1,525	61,585	15,923	7,462	1,274	0,597	21,19	24,46
13-07-2004	starvation	starva 3	21,9	133,24	1,269	31,911	9,809	7,362	0,654	0,491	20,74	20,67
13-07-2004	starvation	starva 4	22	150,19	1,410	39,967	8,512	5,667	1,055	0,702	20,96	24,66
13-07-2004	starvation	starva 5	20,7	115,01	1,297	32,106	4,754	4,134	0,548	0,476	20,60	19,76
26-07-2004	control	control 9	24,5	215,22	1,463	18,809	15,088	7,011	2,646	1,229	23,47	27,82
26-07-2004	control	control 4	23,4	172,98	1,350	20,232	9,919	5,734	1,872	1,082	24,64	36,30
26-07-2004	control	control 7	23,5	187,14	1,442	21,478	11,641	6,220	1,144	0,611	23,74	31,23
26-07-2004	control	control 13	25,0	226,20	1,448	39,019	13,021	5,756	1,705	0,754	24,61	23,71
26-07-2004	control	control 12	22,8	177,17	1,495	37,242	7,561	4,268	1,369	0,773	23,22	45,77
26-07-2004	starvation	starva 5	20,0	132,02	1,650	32,898	7,973	6,039	0,725	0,549	23,69	24,90
20-07-2004	starvation	starva 8	19,1	93,04	1,335	21,542	4,193	4,507	0,605	0,650	22,10	20,45
20-07-2004	starvation	starva 14	20,5	121,65	1,412	29,564	6,159	5,063	0,966	0,794	23,64	23,07
26-07-2004	starvation	starva 15	20,5	103.02	1,403	29,711	5 000	5,197	1,384	1,145	22,09	23,03

Tabel I: Rådata for målinger på hele fisk, filet, indvolde og lever. Control = fodrede fisk Starvation = fisk sultet fra 15. april 2004.







Fillet dry weight %





Mesenteric wet weight



Figur 2.

Fish wet weight





Condition (Fulton K)





Mesenteric weight % of wet weight







Figur 9.

Liver dry weight %



Figur 8.



Liver weight % of wet weight

Allocation of lipid in wild Eurasian perch (*Perca fluviatilis*) in autumn and spring.

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INTRODUCTION

Aquaculture represents an increasing part of the world's fisheries production, today amounting to 16 million tons fish per year, corresponding to 20% of the total production (Danish Institute for Fisheries Research^a: http://www.dfu.min.dk). During the last fifteen years there has been an increasing interest in Eurasian perch *Perca fluviatilis* as a consumption fish in Europe, and perch is now a commonly used fish for freshwater aquaculture (Mélard *et al.*, 1996; Xu *et al.*, 2000; Mathis *et al.*, 2003). Still, Eurasian perch is a relatively new species in aquaculture, and there has been minimal investigation into their nutritional requirements (Brown *et al.*, 1996).

Lipids provide the most economical form of energy storage, and fish usually store excess energy as lipid (Guijarro *et al.*, 2003). Allocation and amount of lipid storage in fish varies with season and feeding, but there is also variation between species, and with size within a given species (Tarr, 1969; Henderson & Tocher, 1987; Storebakken *et al.*, 1991; Shearer, 1994). The liver is important as lipid storage in marine fish. On the contrary lipid rich livers are not found in freshwater species, such as perch, under naturally nutritional and environmental conditions (Henderson & Tocher, 1987). Salmonids deposit storage lipid in their muscles, whereas most whitefleshed fish, like perch, deposit little or no lipid in their muscles (Tarr, 1969; Xu *et al.*, 2001; Jobling & Johansen, 2003). In fish, as in mammals, adipose tissue is considered to function in storage of lipid. In salmonids the adipose tissue associated with the gut and gonads are thought to have this function (Henderson & Tocher, 1987). The same is likely to be the case in perch, but it was not possible to find any studies on this particular species.

Eurasian perch is a common species in Danish freshwater. Under natural conditions, perch is a shoaling, opportunistic predator, which feed on prey ranging from zooplankton and benthic invertebrates to fish (Thorpe, 1977; Jacobsen et al., 2002). Since perch is a carnivorous fish it has a high protein requirement (Fiogbé et al., 1996). Diets for perch in aquaculture must therefore contain an adequate amount of protein to sustain growth, but excessive protein does not bring any further growth benefit (Koskela, 1995). Because protein is the most expensive part of the fish diets any reduction in dietary protein, without negatively affecting fish growth, can substantially reduce the cost of feeding the fish in aquaculture (Fiogbé et al., 1996). Another way of reducing expenditure is by increasing the amount of fat in the diet. This could be done as long as the protein content is kept at a reasonable level. Increasing lipid content would increase the possibility that dietary protein would not be used for energy, but for the growth and tissue production, leading to faster growth (Xu et al., 2001). This has been shown in salmonids (Cho, 1992; Hillestad & Johnsen, 1994; Shearer, 1994) as well as in juvenile Eurasian perch (Kestemont et al., 2001; Xu et al., 2001). Since perch is a high value fish even a small increase in fillet yield would result in a substantial increase in income (Bosworth et al., 1998). Several other beneficial effects of the high-energy versus low-energy diets such as: feed efficiency, protein utilization and reduced nutrient discharges, have been documented (Cho, 1992; Hillestad & Johnsen, 1994; Kestemont et al. 2001; Xu et al., 2001). If however the lipid percent of the diet exceeds a certain level it may impair growth, feed efficiency and liver function in Eurasian perch (Xu et al., 2000). This could contribute to unexplained mortality in perch aquaculture (Kestemont et al., 1997 in Mathis et al., 2003). However, a feeding experiment with juvenile Eurasian perch revealed that increasing dietary lipid content caused increased growth, feed efficiency and protein utilization of the perch, but did not affect mortality (Xu et al., 2001). There is no consensus about the connection between fish mortality and lipid in diet.

Higher total lipid levels in cultured fish, compared to wild fish, are a common phenomenon and are observed for a variety of species (Wood *et al.*, 1957; Flood *et al.*, 1996; Sérot *et al.*, 1998; Grigorakis *et al.*, 2002). Excessive liver lipid, as a consequence of high lipid content in the diet, would be expected to influence liver function (Flood *et al.*, 1996). It has been observed that several



species may increase flesh lipid content when fed high-energy diets, and this may affect flesh quality (Flood *et al.*, 1996; Einen & Roem, 1997). Another problem with high lipid content in the diet is that the fish will deposit excess fat in the visceral tissue, which leads to a decrease in slaughter value (Danish Institute for Fisheries Research^b: http://www.dfu.min.dk). The excess visceral fat could in addition potentially threaten the health of the fish. There is a growing interest in evaluating the effects of dietary factors on chemical composition, flavour, texture, flesh quality and health conditions of Eurasian perch (Xu *et al.*, 2000; Facultes Universitaires Notre-Dame de la Paix: http://www.fundp.ac.be).

An experimental aquaculture project on Eurasian perch was started on Bornholm, Denmark in May 2003. The aim of the project was to examine the possibility of establishing a commercial perch aquaculture in Denmark (Danish Ministry of Food, Agriculture and Fisheries: http://www.fvm.dk). When the perch in the experimental aquaculture reached the minimum commercial size of 100 g, they started dying in a small number each day. The dead perch were tested and found to be clear of viral, bacterial and parasitic infections. Water quality was also found to be fine. This narrowed the cause of death down to the possibility of a nutritional problem. A preliminary examination of the perch revealed a large amount of lipid deposited in the visceral tissue, which indicated that the problem was related to the amount of lipid in the diet and/or the feeding regime. After initiation of this study the feeding frequency was changed from every day to every other day. Following this shift in feeding regime the mortality of the perch decreased, which could indicate that the feed ration had been too high and may have caused the unexplainable deaths, but this has not been investigated further.

The aim of this study was to compare the Eurasian perch from the experimental aquaculture on Bornholm with wild Eurasian perch and see if there was a difference in the allocation and amount of lipid in the liver, muscle, viscera and gonad. Several other parameters such as: sex, total length, total weight, weight of gonads, hepato-somatic index (HSI) and the water percent of the liver, were also examined. In addition differences between perch from spring and autumn were investigated. We did not expect the muscle lipid to vary between wild and cultured perch, since muscle tissue is not engaged in adiposity in perch. On the contrary we expected to see a higher amount of lipid in the visceral tissues and livers of the cultured perch, compared to the wild perch, since it was assumed that the controlled diet of the cultured perch contained more lipid than the uncontrolled diet of the wild perch. If this was found, it could be a result of diet or other rearing conditions, and could possible cause the unexplained mortality in the experimental aquaculture.

MATERIALS AND METHODS

SAMPLING

Cultured Eurasian perch was obtained from the experimental aquaculture facility on Bornholm. The perch were cultured at a temperature of 17-20 °C and with a photoperiod of 18L:6D. After hatching the perch were fed a dry pellet cod feed (DAN-EX 1362) with a lipid content of 13% and a protein content of 62%. Five months after the hatching, the diet was switched to another cod feed (DAN-EX 1344) containing a lipid content of 13% and a protein content of 44%. Each day the perch were fed 2-5% of their body weight, the amount was decreased as the perch were growing. The cultured perch were hatched from eggs harvested in Tange Sø (a Danish fresh water lake). Autumn perch (CA) were sampled in September 2003 and stored at -25 °C until analysed. The age was 4 months, the length was 13.19 ± 0.70 cm (mean ± S.D.) and the weight was 36.08 ± 5.02 g (mean ± S.D.). Spring perch (CS) were sampled in April 2004 and stored at -25 °C.



The age was 11 month the length was 20.89 ± 1.40 cm (mean \pm S.D.) and the weight was 125.21 ± 29.48 g (mean \pm S.D.).

Wild Eurasian perch were obtained from Tange Sø. The water temperature of the lake was approximately 4 °C in November 2001 and 8 °C in April 2001 (Gudenåcentralen: www.gudenaacentralen.dk). Autumn perch (WA) were caught in October 2003. They were stored at -80 °C for five months, and then transferred to a -25 °C freezer until analysed. The age was estimated to approximately 6-7 years (Danish Institute for Fisheries Research^c: www.dfu.min.dk), the length was 25.54 ± 2.08 cm (mean \pm S.D.) and the weight was 243.51 ± 65.34 g (mean \pm S.D.). Spring perch (WS) were caught in April 2004. They were stored at -25 °C. The age was estimated to approximately 7-8 years (Danish Institute for Fisheries Research^c: www.dfu.min.dk), the length was 28.05 ± 1.42 cm (mean \pm S.D.) and the weight was 300.95 ± 42.98 g (mean \pm S.D.).

Ten perch were from each of the four groups were analysed. The length of each fish was measured from the snout to the tip of the tail (total length). Total weight was measured after surface water was wiped off. Sex was determined when possible and the weight of liver and gonads were measured. Sub-samples for lipid analyses were taking from liver, muscle tissue (from the side fillet), viscera (the sample excluded liver, heart, gonads, stomach content and gut content) and gonads. Samples were homogenised and stored at -25 °C until further analyses. A sub-sample of each liver sample was not frozen, but immediately used to determine water percent.

PROCEDURE

Water percent in liver was measured by oven drying the weighed sub sample of the homogenised liver for 24 hours at 60 °C and then reweighing the dried liver sample.

Total lipid in liver, muscle tissue, visceral tissue and gonads was measured gravimetrically according to the method of Bligh and Dyer (1959). The homogenised tissue samples were weighed out with four decimals. The amount of sample from each tissue was kept constant as far as possible. Liver sample: 0.5 g, muscle sample: 3.0 g, visceral tissue sample: 3.0 g and gonad sample: 2.5 g. In some of the cultured perch from the autumn, the livers weighted less than 0.5 g therefore smaller samples were used. The optimal sample size is between 1 and 5 g for fish muscle (Honeycutt et al., 1995). It was not possible to find guidelines for the optimal sample amount of the other tissues. The lipid extraction was performed in fume cupboard. Ion exchanged water was added to make a total of 20 ml water including the water present in the sample. 50 ml methanol (CH₃OH, clean, density 0.97 g/ml) was added and the sample was placed in iced water. 25 ml chloroform (CHCl₃, clean, density 1.47 g/ml) was added and the sample was mixed with an Ultra Turrax (T25, IKA Labortechnik, Staufen, Germany) for 1 min. A further 25 ml chlorofom was added and the sample was mixed for another 1 min. Then 25 ml ion exchanged water was added and the sample was mixed for 5 sec. To obtain complete separation of the two phases the mixture was centrifuged for 10 min. at 2900 rpm in a thermostatic centrifuge (Sorvall RC 5C plus, rotor SLA-1500). Temperature in the centrifuge was kept at 4 °C. The upper methanol/water layer was removed and 10 ml of the lower organic chloroform/lipid layer was transferred to a pre weighted aluminium tray with a pipette. After the chloroform had evaporated (75 min.) the sample was dried for 30 min. at 105 °C and exact weight was recorded. The sample was kept on ice during the experiment to avoid extensive oxidation of lipids. For instrument arrangement see enclosure 1.



(1)

Lipid content (%) =
$$\frac{(gl * 50 * 100)}{((ex * (gl / 0.9)) * gs)}$$

gl = gram lipid in the sample
ex = ml of the chloroform/lipid phase
gs = g sample tissue
50 = ml of chloroform added
0.9 = the specific gravity of the lipid (table value)

Hepato-somatic index (HSI) (%) =
$$\left(\frac{\text{liver wet weight (g)}}{\text{body wet weight (g)}}\right) * 100$$
 (2)

STATISTICAL ANALYSIS

To examine differences in the hepato-somatic index a one-way ANOVA was used, and *post hoc* tests (LSD) was used to locate the differences between groups. The same procedure was applied on the allocation of lipid in the liver, muscle, visceral tissue and hard and soft roe. Simple linear regression was used to relate lipid content in the liver, muscle and visceral tissue to fish length and weight. Simple linear regression was also used to determine if there was a relationship between liver lipid percent and liver water content. Independent samples t-test was used to evaluate the relationship between sex in addition to autumn versus spring perch, and cultured versus wild perch. All statistical significance was $P \le 0.05$. Statistical analyses of the data were performed using SPSS 11.0 for Windows.

RESULTS

To test the method, four identical samples of the cod feed (DAN-EX 1344), with a declared lipid percent of 13% were analysed. The result was a mean lipid percent of $13.12 \pm 0.08\%$ (mean \pm SE) calculated from equation (1).

CORRELATIONS BETWEEN FISH LENGTH, WEIGHT AND LIPID ALLOCATION

One-way ANOVA showed that there was significant difference between the log-transformed lengths of the four groups (CA, CS, WA, WS) (ANOVA, $F_{3.36} = 267.74$, P < 0.001). *Post hoc* tests (LSD) showed that the mean length of each of the four groups differed from all of the others (Fig. 1a). One-way ANOVA showed that there was also significant difference between the log-transformed weights of the four groups (CA, CS, WA, WS) (ANOVA, $F_{3.36} = 208.85$, P< 0.001). *Post hoc* tests (LSD) showed that each of the four groups differed from all of the others in weight (Fig. 1b).

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Fig. 1. (a) Total length and (b) total weight of Eurasian perch in the groups, given by mean \pm S.D. (CA: Cultured, autumn, CS: cultured, spring, WA: wild, autumn, and WS: wild, spring). Significant difference is indicated as *, P < 0.05. n = 10.

There was no linear relationship between lipid content in liver, muscle or viscera and length or weight of the perch in any of the four groups (ANOVA, P > 0.05). There seemed however to be an overall tendency for the longer perch to have a smaller amount of liver lipid (Fig. 2a). The amount of fat in the muscle tissue was fairly constant among the groups and was likely to be independent of fish length (Fig. 2b). An overall tendency for the longer perch to have a smaller amount of lipid in the visceral tissue was found (Fig. 2c). In the following the tendency towards a correlation between lipid percent in tissue and fish length is neglected. Furthermore there was an overall tendency for the heavier perch to have a smaller amount of liver lipid (Fig. 2d). The amount of lipid in the muscle tissue was fairly constant among the groups and was likely to be independent of fish weight (Fig. 2e). There was a great variation of lipid percent in visceral tissue but a small tendency for the heaviest perch to have a lower amount of visceral fat was observed (Fig. 2f). In the following the tendency towards a correlation between lipid percent in visceral tissue but a small tendency for the heaviest perch to have a lower amount of visceral fat was observed (Fig. 2f). In the following the tendency towards a correlation between fish weight and lipid percent in liver or visceral tissue were neglected.





Fig. 2. Correlation between length and (a) liver lipid percent, (b) muscle lipid percent, (c) visceral lipid percent and correlation between weight and (d) liver lipid percent, (e) muscle lipid percent, (f) visceral lipid percent. Scatter plots showed no significant correlation in any of the cases P > 0.05. n = 8-10.



SEX

Sex distribution in the four groups is seen in Table 1. It was impossible to determine the sex of some of the immature, cultured perch from autumn and one of the wild perch. To test if there was a correlation between sex and the measured parameters (liver lipid, muscle lipid, visceral lipid, total weight and total length) an independent-samples t-test was used on the groups CS and WS. These were the only groups, in which sex could be determined for all the perch. The tests showed no significant difference between females and males in the two groups except for the muscle lipid in CS (t-test, t = 2.83, P = 0.022). We chose to ignore this one aberration and assumed that there was no correlation between sex and the allocation of lipids; hence the sexes were pooled in the following analyses. The gonads are a special case that will be discussed separately.

 Table 1. Sex distribution of Eurasian perch in the groups. (CA: Cultured, autumn, CS: cultured, spring, WA: wild, autumn, WS: wild, spring).

 Undetermined sex is marked with ? n = 10.

	9	2	?
CA	4	2	4
CS	7	3	0
WA	9	0	1
WS	4	6	0

LIPID PERCENT IN LIVER, MUSCLE AND VISCERA

Owing to technical difficulties there were only 9 liver samples for lipid extraction in the group CS. Furthermore one value of liver lipid percent in the group CS differed considerably from the rest and was neglected. There was significant difference in the liver lipid percent among the four groups (CA, CS, WA, WS) (ANOVA, $F_{3.34} = 84.84$, P< 0.001). *Post hoc* tests (LSD) showed that each of the four groups differed from all of the others (Fig. 3). The tendency was that the cultured perch had a higher amount of liver lipid than the wild perch. To examine the difference in lipid content in liver tissue between cultured and wild perch the groups CA and CS, and WA and WS were split into two groups: cultured (CA, CS) and wild (WA, WS). T-test showed that the cultured perch had significantly more liver lipid than the wild perch (independent-samples t-test, t = 6.16, P < 0.001) (Fig. 4).

No significant difference in the muscle lipid percent among the groups was found (ANOVA, $F_{3..36} = 1.55$, P = 0.22) (Fig. 3). To examine the difference in lipid content in muscle tissues between cultured and wild Eurasian perch the groups CA and CS, and WA and WS were split into two groups: cultured (CA, CS) and wild (WA, WS). T-test showed that the muscle lipid percent was not significantly different between the cultured and wild perch (independent-samples t-test, t = 1.67, P > 0.05) (Fig. 4).

Owing to technical difficulties there were only 9 visceral samples for lipid analyses in the group CS. There was significant difference between the visceral lipid percent of the four groups (ANOVA, $F_{3.35} = 14.16$, P < 0.001). *Post hoc* tests (LSD) showed that the two groups of cultured perch had significantly more visceral fat than the two groups of wild perch (Fig. 3). To examine the difference in lipid content in visceral tissues between cultured and wild Eurasian perch the groups CA and CS, and WA and WS were split into two groups: cultured (CA, CS) and wild (WA, WS). T-test showed that the cultured perch had significantly more visceral lipid than the wild perch (independent-samples t-test, t = 5.88, P < 0.001) (Fig. 4).





Fig. 3. Lipid percent of the tissues in the groups of Eurasian perch, given by mean \pm S.E. (CA: Cultured, autumn, CS: cultured, spring, WA: wild, autumn, WS: wild, spring). Points from the same tissue, marked with the same letter are not significantly different, P > 0.05).



Fig. 4. Lipid percent in the different tissues of Eurasian perch in the groups: cultured (CA+CS) and wild (WA+WS). Values are given by mean ± S.E. Significant difference between the same tissue in different groups is indicated as *, (P < 0.05). n = 8-10.

GONADS

It was impossible to test differences in gonad lipid between the groups without dividing into soft roe and hard roe, since there were a varying number of males and females in the four groups. Univariate analysis of variance showed no significant difference in the lipid percent in the female hard roe of the three groups (CS, WA, WS) in which hard roe was found (one-way ANOVA, $F_{2.17}$ = 0.74, P = 0.49). Likewise univariate analysis of variance showed no significant difference in the lipid percent in the male soft roe of the two groups (CS, WS) in which soft roe was found (one-way ANOVA, $F_{1.5}$ = 3.65, P > 0.05).

There was significant difference between the lipid percent of the male soft roe and the female hard roe in the group WS (independent-samples t-test, t = 8.03, P < 0.001). The lipid percent in the hard roe was twice as high as the lipid percent in the soft roe. In the group CS, there was no significant difference between lipid percent in hard and soft roe (independent-samples t-test, t = 1.34, P > 0.05).

The data range was too small to test any variation in gonad weight, but the data indicated a small trend towards heavier gonads in the female individuals of the spring groups compared to the autumn groups.

HEPATO-SOMATISK INDEX

There was significant variance in the hepato-somatic index (HSI) between the four groups (CA, CS, WA, WS) (ANOVA, $F_{3,36} = 14.39$, P < 0.001). *Post hoc* tests (LSD) showed that each of the four groups differed from all of the others, except the groups CS and WS, which were not significantly different from each other. In autumn the cultured perch had a significantly lower HSI than the wild perch. The opposite was the case for the spring perch, but it was not significant. HSI was highest in autumn for both wild and cultured perch (Table 2).

 Table 2. Hepato-somatic index (HSI) in Eurasian perch is calculated from equation (2). (CA: Cultured, autumn, CS: cultured, spring, WA: wild, autumn, WS: wild, spring.). (Mean ± S.D.), n = 10.

Fish group	HIS (%)
CA	1.52 ± 0.43
CS	1.04 ± 0.26 ^a
WA	1.87 ± 0.30
WS	$0.97 \pm 0.40^{\text{ a}}$

Values marked with the same letter are not significantly different (P > 0.05).

RELATIONSHIP BETWEEN WATER PERCENT AND LIPID PERCENT IN LIVER

One-way ANOVA showed that there was an inverse linear relationship between the liver lipid percent and the water percent in the liver, in perch from the groups CA, CS and WS (ANOVA, $F_{1.8}$, P < 0.05), but not for the group WA (ANOVA, $F_{1.6} = 0.02$, P = 0.88). The tendency was that the water content was smaller in the more fatty livers (Fig. 5).



Fig. 5. Relationship between liver lipid percent and water percent in the liver of Eurasian perch. Scatter plots show significant correlation for the groups, CA, CS and WS, (P < 0.05). No relation was found in the group WA, (P > 0.05). n = 8-10.

AUTUMN VERSUS SPRING

To examine a possible relationship between lipid allocation and season, the four groups CA and WA, and CS and WS were divided in to two groups: autumn (CA, WA) and spring (CS, WS). There was no significant difference between muscle lipid in the autumn and spring groups (independent-samples t-test, t = 1.26, P > 0.05). The same was found for the visceral lipid (independent-samples t-test, t = 1.44, P > 0.05). Significant difference was found for liver lipid between the autumn and spring perch (independent-samples t-test, t = 2.51, P < 0.001) (Fig. 6).



Fig. 6 Lipid percent in the liver, muscle and visceral tissue in Eurasian perch, in the groups: autumn (CA+WA) and spring (CS+WS). Values are given by mean ± S.E. Significant difference between the same tissue in different groups is indicated as *, (P < 0.05). n = 8-10.

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DISCUSSION

It was not possible to attain wild Eurasian perch in the same size (and age) as the cultured perch. The wild perch were significantly longer, heavier and older than the cultured perch and this must be considered a source of error in our results. When comparing cultured perch with wild perch and autumn perch with spring perch the original four groups was pooled into two heterogenic artificial groups. The results gained by this pooling could be a result of the heterogenic groups rather than actual environmental and feed associated factors.

LENGTH, WEIGHT AND AGE

The difference in total weight and total length, between the wild Eurasian perch from autumn and spring, could be due to natural variation in the population (the sample size is relatively small). The difference between the cultured perch from autumn and spring is presumably due to the age difference (4 versus 11 month).

The growth constant K from the von Bertalanffy equation range between 0.09 and 0.76 for Eurasian perch (http://www.fishbase.org). This indicates a great plasticity in growth rate in perch. This plasticity is influenced by for instance temperature (Karås & Thoresson, 1992; Mélard *et al.*, 1996). There is a positive relationship between water temperature and growth of Eurasian perch (Karås & Thoresson, 1992; Mélard *et al.*, 1996) as well as rainbow trout *Oncorhynchus mykiss* (Cho, 1990). Mélard *et al.* (1996) found that the maximum growth for Eurasian perch occurred at 23 °C. Other factors may affect the growth as well, for instance food availability (Fontaine *et al.*, 1997) and genetic traits (Brown, 2002). Genetically differences should be ruled out in this experiment since all the perch originated from Tange Sø.

Eurasian perch is a slow growing fish and even in optimal rearing conditions it seldom exceeds 120 g bodyweight in the first year (Mélard *et al.*, 1996). On this basis it can be concluded that the growth of the cultured perch in this experiment was near the optimal, since the weight of the 11 months old cultured perch (CS) was $125,01 \pm 29,48$ g (mean \pm SD). This is in agreement with the finding that a dietary lipid concentration of 12-15% induces maximum growth in Eurasian perch (Kestemont *et al.*, 2001) and the fact that the cultured perch in this study was fed a diet containing 13% lipid. It can be assumed that the wild perch have a slower growth rate than the cultured perch in the present study, because the cultured perch had higher food availability and were presented with higher winter temperatures than the wild perch. In addition it is likely that cultured perch is less active than wild perch, which leaves them with a higher amount of excess lipid, since lipid is metabolized during exercise in fish (Kieffer *et al.*, 1998).

No significant relationship was found between total length or total weight and the measured variables. This lack of correlation between length or weight and adiposity in the present study, could be due to limited sample size. On the other hand the same pattern was seen on total body lipid in a study with sea bream *Sparus aurata* (Grigorakis *et al.*, 2002). There was however a tendency for the longer and heavier perch to have a smaller amount of lipid in liver and visceral tissue. This could be an artefact because the sample groups are very heterogeneous in age, size and rearing conditions. All the longest and heaviest perch were wild and had been exposed to other biotic and abiotic factors than the smaller cultured perch. This may have influenced the comparisons in the present study. It is normally a general pattern that total body lipid increases with fish size in a variety of species, European ell *Anguilla anguilla* (Henderson & Tocher, 1987), blunt nose minnow *Pimephales notatus* (Henderson & Tocher, 1987) and salmonids (Ayles, 1975; Storebakken *et al.*, 1991; Shearer, 1994).



The lipid percent in mature perch of both sexes is consistently lower than that of immature perch, because of the utilization of lipid stores for gonad production (Henderson & Tocher, 1987). The perch in the group CA were likely to be immature and this might have interfered with the results. Roe was found in the 11 months old perch in the group CS, which is a sign of maturity. This is inconsistent with the norm that wild male Eurasian perch mature at ages 2-3 years, and females at ages 3-6 years (15-25 cm) (Tesch, 1955 in Thorpe, 1977; Danish Institute for Fisheries Research^c: http://www.dfu.min.dk). The early maturity could be due to the fact that lipid may be a prerequisite for the hormonal triggering of sexual maturation (Rowe *et al.*, 1991). It could also be imagined that the cultured perch grows fast and reach the minimum size for maturity faster than the wild perch.

DIET

The diet is considered an important factor in the growth, health, and consumption value of reared fish (Brown *et al.*, 1996; Fontaine *et al.*, 1997; Xu *et al.*, 2001). Few nutritional requirements have been established for yellow perch, thus there is no diet formulated specifically for this species (Brown *et al.*, 1996). As far as we know this counts for Eurasian perch as well. The current recommendation is use of feeds formulated for trout and salmon (Brown *et al.*, 1996), but the cultured fish from Bornholm were fed a cod diet. Yellow perch *Perca flavescens* is a leaner fish than salmonids, and has a content of muscle fat close to 1% compared to about 4% for wild salmonids (Henderson & Tocher, 1987). Yellow perch is very closely related to the Eurasian perch (Thorpe, 1977) so it can be assumed that the same counts for Eurasian perch. Atlantic cod *Gadus morhua* is a lean predatory fish like the perch, with a muscle lipid percent below 1% (Jensen *et al.*, 2003). This makes it reasonable to feed the Eurasian perch with cod feed instead of feed formulated for salmonids.

The cultured perch were fed a diet with a lipid content of 13% and a protein content of 44-62%. Dietary lipid content of 12 to 15% is recommended for Eurasian perch (Kestemont *et al.*, 2001) and a minimum of 36% dietary protein is necessary for optimal growth of Eurasian perch (Fiogbé *et al.*, 1996) and yellow perch *Perca flavescens* (Brown *et al.*, 1996). Each day the cultured perch were fed 2-5% of their body weight. 3% was recommended in a study on yellow perch (Brown *et al.*, 1996). Thus, compared to other studies the diet and feeding regime of the cultured perch seem reasonable. It should however be possible to decrease the protein level for economical reasons. As mentioned before the feeding frequency in the experimental aquaculture on Bornholm has been changed from every day to every other day. After this shift in feeding regime the mortality of the perch decreased, which could indicate that the food ration had been too high and may have caused the unexplainable deaths.

In contrast to the known diet of the cultured perch there was no control or examination of the diet eaten by the wild perch. It was assumed that the diet of the wild perch contained a lower level of lipid and that the overall energy intake of the wild perch was lower than that of the cultured perch.

LIPID PERCENT IN LIVER, MUSCLE AND VISCERA

Under normal conditions the liver is not used for lipid storage in freshwater fish (Henderson & Tocher, 1987). In a study on wild Eurasian perch a liver lipid content of 3.1% was found (Gunstone *et al.*, 1978 in Henderson & Tocher, 1987) compared to 67% in cod *Gadus morhua*; that is a marine species (Jangaard *et al.*, 1967 in Henderson & Tocher, 1987). The wild perch in the present study had a liver lipid content of $4.50 \pm 0.21\%$ (mean \pm S.E.) which correlates with the result of Gunstone *et al.* (1978). The lipid content was significantly higher in the cultured perch (8.71 \pm 1.23% (mean \pm S.E.)), which could indicate differences in rearing conditions (Henderson & Tocher, 1987).



The liver lipid accumulation in perch is affected by protein/energy ratios in the diet (Xu *el al.*, 2001) and could be a contributing factor in the unexplained mortality in perch aquaculture (Brown *et al.*, 1996; Xu *et al.*, 2000). Higher liver lipid percent in the cultured perch, compared to the wild perch was found in this study (Fig. 4). With few exceptions, this also applies for salmonids (Wood *et al.*, 1957). Furthermore it was observed that a few of the cultured perch had enlarged livers that were paler than normal; this may indicate a feed imbalance (Hillestad & Johnson, 1994). In an eight week feeding experiment with Eurasian perch, fed a diet containing 40% protein and respectively 6%, 12% or 18% lipid, ultra structural observations were performed on the liver tissue. The finding was that the group fed the 18% dietary lipid had a high accumulation of large lipid droplets in the hepatocytes inducing a marked reduction of mitochondria and rough endoplasmic reticulum compared with the other groups. This can be considered a preliminary sign of impaired liver function. It was assumed that a diet with lower lipid content could cause the same effect if the experiment was extended to a longer period (Kestemont *et al.*, 2001). Without ultra structural observations it is impossible to make a final conclusion on the condition of the livers in this experiment.

Impaired liver function, for instance lipoid liver disease (fatty liver), is not only considered a result of excess lipid in the diet. Another cause is rancid fat (coursed by oxidation of dietary lipid) in the diet (Cho, 1990; Kestemont *et al.*, 2001). This can be avoided by storing the feed in a cool place (Cho, 1990; Jessen & Nielsen, 2002).

In the present study there was no significant difference in muscle lipid percent between the four groups (Fig. 3). There was therefore no significant difference in muscle lipid percent between the cultured and wild perch (Fig. 4). This suggests that the lipid content in the muscle tissue may not be affected by the factors that separate the wild and cultured perch (e.g. food, activity). This is in agreement with a feeding experiment on juvenile Eurasian perch where no significant adiposity in the muscle tissue was found (Xu *et al.*, 2001). The average value of muscle lipid in this study was $1.01 \pm 0.06\%$ (mean \pm S.E.). This correlates with earlier results, showing that wild yellow perch *Perca flavescens* is a lean fish with muscle lipid content close to 1% (Henderson & Tocher, 1987). Generally muscle tissue in perch is not involved in energy storage (Shearer, 1994). This is in contrast to salmonids were the muscle tissue played an important role in energy storage (Jobling & Johansen, 2003).

Lipid content is an important feature of fish quality (Flood *et al.*, 1996; Sheehan *et al.*, 1996; Einen & Roem, 1997) since it influences on the taste and applications of the flesh (e.g. smoking). Furthermore an eventually increase of lipid in edible muscles of cultured fish can presents a problem as it can serve as a storage depot for unmetabolized, potentially harmful, fat-soluble pollutants (Flood *et al.*, 1996). Since no difference in muscle lipid was found in the present study or in a study on Eurasian perch by Xu *et al.* (2001), this should not be a concern in rearing of Eurasian perch. Great differences exist between artificial diets of hatchery fish and natural food of wild fish, and these differences in nutrition directly influence the composition and condition of the fish (Wood *et al.*, 1957). This means that the fatty acid composition may be affected by the lipid pattern of the diet (Wood *et al.*, 1957; Henderson & Tocher, 1987; Sérot *et al.*, 1998). Even though there was no difference in lipid content in muscles between the wild and cultures perch in the present study (Fig. 4), there could possible be a difference in lipid composition. This could affect nutritional value of the fish.

Another important consumption quality factor is the flesh texture. The cultured perch were found to have a finer muscle texture than the wild perch, a phenomenon also observed in cultured fish of other species (Aoki *et al.*, 1991). The finer muscle texture could be a result of the faster growth of the cultured fish and might result in less dense fillets which could influence the consumption value (Danish Institute for Fisheries Research^d: http://www.dfu.min.dk).



The main storage area for lipid in perch is the visceral tissue (Shearer, 1994). The amount of lipid in the visceral tissue could be related to the dietary lipid content since adiposity is affected by dietary manipulations (Shearer, 1994). The proportion of fat in the viscera of Eurasian perch has been reported to increase significantly as the concentration of fat in the diet is increased (Xu *et al.*, 2001). In the present study it was found that the cultured Eurasian perch had more visceral lipid than the wild perch (Fig. 4). This could indicate a difference between life conditions for cultured and wild perch. The same pattern was found in rainbow trout *Oncorhyncus mykis* (Jobling *et al.*, 1998) and in Atlantic salmon *Salmo salar* (Hillestad & Johnsen, 1994; Einen & Roem 1997). It was not possible to find any studies on the health effects of excess adiposity in the visceral tissue, but if the amount of lipid is as high as in the cultured perch in this study, it must be assumed to have an influence on the fish.

GONADS

The wild perch in this study had not yet spawned when they were caught in the beginning of April, but were due to spawn the following weeks. Danish perch generally spawns in April (Muus & Dahlstrøm, 1998). The cultured perch from spring was sampled in April and should have spawned, but they had not yet developed fully mature gonads. The reason for this was probably their young age and the fact that they had not been exposed to a low winter temperature, which is thought to be important for the triggering of spawning (personal comment Julia Lynne Overton).

No variation in gonad lipid percent was found within the separate sexes. That is, no difference was found between wild or cultured perch or between perch from autumn and spring. Normally the lipid content of gonads is highly depended upon the stage of the yearly sexual cycle plus the maturity stage of the fish (Henderson & Tocher, 1987). The reason this was not observed may be due to the limited sample size. A small trend towards heavier gonads in the female individuals of the spring groups, compared to the autumn groups, was however observed.

For a given species the lipid content of the testes is always less than that of the ovary (Henderson & Tocher, 1987). This is found in, for instance, largemouth bass *Micropterus salmoides* (Brown & Murphy, 2004). Significant difference between the lipid percent of the male soft roe and the female hard roe was found in the group WS (with the lipid percent in the hard roe twice the amount of the lipid percent in the soft roe in WS). On the contrary no significant difference was found in the group CS. This aberration may be due to the limited sample size.

HEPATO-SOMATISK INDEX (HSI)

The hepato-somatic index of the liver is partly depended upon the stage of the sexual cycle because liver lipid reserves is used for gonadic development, as seen in for instance tench *Tinca tinca* and Eurasian perch (Henderson *et al.*, 2000; Guijarro *et al.*, 2003, respectively). In the present study the cultured perch from the autumn (CA) had a significantly higher HSI than the cultured perch from the spring (CS). The same pattern was significant for the wild perch (Table 2). As mentioned above it was impossible to test seasonal variation in gonad lipid percent or gonad weight. But the data indicated a small trend towards heavier gonads in the female individuals of the spring groups, which could correlate with the observed decrease in HSI.

CORRELATION BETWEEN LIPID PERCENT AND WATER PERCENT IN LIVER

There was a significant inverse relationship between liver lipid percent and water percent in the liver in CA, CS and WS (Fig 5). A reason that WA did not show a significant relationship between water and lipid content could be that the data range was too small due to the limited sample size. No other studies concerning this relationship were found but the water content of whole salmonids is found to increase as the lipid reserves decreases (Shearer, 1994; Koskela *et al.*, 1998).



If this tendency is constant, and holds for separate tissues, it should be possible to estimate the lipid percent of the liver, or other tissues, from the water content (Shearer, 1994). It would be of interest to exchange the toxic and time consuming Blight and Dyer (1959) lipid extraction with the easier determination of water content. It was however not possible to conclude anything from the present study since the result is not unambiguous.

SEASONS

Temperature and day length influence on the growth and feeding level of reared fish (Karås & Thoresson, 1992; Mélard *et al.*, 1996). Generally species used in aquaculture are only a few generations removed from the wild and this means that the fish are adapted to conditions in the environment in which they evolved (Huntingford & Thorpe, 1990). The cultured perch were reared at a constant "summer" water temperature of 17-20 °C, which could influence on their lipid reserves. In the summer period Atlantic salmon *Salmo salar* from temperate regions increase intake and replenish lipid and protein stores. In the winter food availability is reduced and a mobilisation of lipid takes place to avoid the use of protein for metabolism (Gardiner & Geddes, 1980). On basis of this it could be assumed that cultured fish reared at a constant, high temperature would store lipid reserves year round. Generally lipid is stored at a lower ration at low temperature, than at higher temperatures, but only up to a certain limit and only if food is plenty (Brett *et al.*, 1969 in Shearer, 1994). The cultured perch were presented with a constant day length of 18 hours. In temperate regions the day length varies over the year and this will affect the feeding rate of perch, since perch is a visual feeder (Karås & Thoresson, 1992).

Significant difference was found for liver lipid between the autumn and spring perch but there was no significant difference in lipid percent between the autumn and spring perch in either muscle or visceral tissue (Fig. 6). In summery a high lipid in cultured perch could be due to temperature as well as diet. Though earlier studies have shown, that diet affects adiposity more than abiotic factors such as temperature and pH (Wood *et al.*, 1957). It would be relevant to perform an experiment, to see the difference in adiposity, in Eurasian perch raised at constant temperature compared to perch raised in natural seasonal temperatures in controlled conditions with the same diet.

CONCLUSSION

This study has shown that the muscle tissue of the cultured Eurasian perch was not significantly fattier than the muscles of the wild perch, which is an advance in consumption purposes. The muscles of the cultured perch had a finer texture, which could be a disadvantage. There was a significantly higher amount of lipid in the liver and visceral tissue of the cultured perch compared to the wild perch, but no final conclusion on the influence on health could be made. The higher lipid in viscera and liver in the cultured perch, could eventually be due to higher lipid content in diet, feeding regime or higher year round water temperature. Feeding regime seems to be the most reasonable explanation since the mortality decreased after the feeding frequency was changed to every other day, but it could be a combination of several factors. A more controlled experiment is required before final conclusions can be made.

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Enclosure



Enclosure 1. (a) Addition of chloroform during mixing with Ultra Turrax.
(b) Transfer of the lower lipid/chloroform layer to an aluminium tray. Centrifuge bottles with two phases separated by a protein layer can be seen in the background.

DFU-rapporter – index

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

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