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Betri nýting vatns í bleikjueldi

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Erfðir og eldi

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Report summary



Titill / Title	Betri nýting vatns í systems for Arctic o	•	nt rearing		
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Ágrip á íslensku:	stærð og framleiðsluget vatni. Markmið verkefnis	Vatnsþörf í fiskeldi er óhemju mikil og það sem endanlega takmarkar stærð og framleiðslugetu fiskeldisstöðva er aðgengi að heitu og köldu vatni. Markmið verkefnisins var að prófa ódýra og einfalda leið til þess að draga úr vatnsnotkun í bleikjueldi.			
	Í upphafi verkefnisins var gert var ráð fyrir því að hægt væri að nýta vatn í bleikjueldi fjórfalt betur en nú er gert. Hins vegar kom í ljós að það er hægt að nýta vatnið sjöfalt betur.				
	Niðurstaða þessarar rannsóknar er sú að hægt er að framleiða í kringum sjö sinnum meira af lífmassa í fiskeldi á landi með því vatnsmagni sem notað er í dag. Markmiðum verkefnisins var því náð og gott betur. Til þess að það sé hægt þarf að hafa eftirfarandi atriði í huga:				
	 Mjög mikilvægt er að losa grugg sem fyrst úr vatninu. Því er tromlusía nauðsynlegur búnaður og ber að sía allt vatnið við hvern hring endurnýtingar. Í síunni ætti að notast við 100 µm dúk en hann hreinsar allar agnir sem minnkað geta virkni eldiskerfisins. Nægur straumur verður að vera í eldiskerjunum og æskilegt er að vatnskiptahraði sé ekki minni en 45 mínútur til að tryggja sjálfhreinsun og til að fullnægja súrefnisþörf fiska við mikla þéttni. 				
	en 0,03-0,05 L kg ⁻¹ Lífhreinsirinn sem i	mín ⁻¹ . Hann losar amm	gar endurnýting er meiri oníak úr eldisvökvanum. nnsókn hefur sýnt sig að un hans.		
Lykilorð á íslensku:	Vatnsþörf - Bleikja - End	urnýtingarkerfi			

Skýrsluágrip Matís ohf

Icelandic Food and Biotech R&D

Report summary



Summary in English:

Aquaculture requires large volumes of water are required for aquaculture and the size and production capacity of fish farms is in most places ultimately determined by access to water and geothermal heat. The objective of this project was to reduce water requirements in Arctic charr aquaculture. Through simple reuse of water the plan was to reduce water requirements fourfold compared with standard reference values in Arctic char fish farms in Iceland. This goal was achived and at the end the reuse was sevenfold.

The conclusions of the project are that by using the same amount of water used today and with a simple reuse of it the annual increase in production of Arctic char can be sevenfold the annual production of today. But to make that possible, the following points have to be kept in mind:

- It is necessary to minimize the turbidity in the water with all means. A drum-filter of 100 μm is therefore needed in the recirculation system.
- The current in the rearing system has to be sufficient and the water change ratio should not be less then 45 minutes to secure self cleaning and to fulfil the oxygen need of the fish reared in high density.
- A bio filter is needed if the recirculation exceeds 0,03-0,05 L kg⁻¹·mín⁻¹. It phases out the ammonia in the rearing system. The bio filter used in this project has shown that it works and the design of it has a patent

English keywords:

Water demand – Arctic char – Recirculation system

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Efnisyfirlit

1.	Inngangur	1
N	Лarkmið og framtíðarsýn	
Т	æknileg markmið	2
٧	'iðskiptaleg markmið	3
2.	Aðferðir	4
E	ldistilraun I	4
E	ldistilraun II	5
3.	Niðurstöður	7
E	ldisrými	7
	Loftari og lágþrýstings súrefniskerfi	7
٧	/axtarmælingar	10
	Eldistilraun I – endurnýting án lífhreinsis	10
	Eldistilraun II	15
4.	Umræður	16
5.	Þakkir	16
6.	Viðaukar	17

1. Inngangur

Vatnsþörf í fiskeldi er óhemju mikil og aðgengi að heitu og köldu vatni er sá þáttur sem takamarkar stærð og framleiðslugetu fiskeldisstöðva. Vatnmagn í meðal ferskvatnslind er á bilinu 10-200 lítrar á sekúndu. Lindir sem eru það gjöfular að gefa 200 sekúndulítra eru mjög sjaldséðar. Lindir eru gjöfulastar á svæðum með yngri bergrunni í svokölluðum Kvarter berglögum sem eru á virku gosbeltunum. Dæmi má nefna Reykjanes, efri hluta Suðurlands og í Þingeyjarþingi. Lindirnar eru oft við yfirborð en eru þaktar jarðvegi og setlögum. Vatnsmagn linda af þessu tagi er um 5-100 l/s, vatnasviðið er oft stórt og magn vatnsins stöðugt [Freysteinn Sigurðsson o.fl. Samorka 2001]. Samanlagt ferskvatnsmagn sem íslenskar fiskeldisstöðvar hafa yfir að ráða eru um 3.000 sekúndulítrar og framleiðslugeta þeirra 870 tonn á ári miðað við að hægt sé að framleiða um 300 kg á ári á hvern sekúndulítra. Samanlagt sjómagn sem landeldisstöðvar hafa yfir að ráða (úr borholum) er um 10.000 sekúndulítrar og framleiðslugeta um 3.600 tonn, og því er framleitt um 360 tonn á ári á hvern sekúndulítra.

Ár	Landið allt	Almennings- vatnsveitur	Landbúnaður	lðnaður	Heimili
	[milj. m³]	[milj. m³]	[milj. m³]	[milj. m³]	[milj. m³]
1980	100	84	4	10	2
1985	103	87	4	10	2
1992	164	82	70	10	2
1993	164	82	70	10	2
1994	164	82	70	10	2
1995	164	82	70	10	2

Tafla 1: Vatnsþörf á Íslandi eftir notendum

Í töflu 1 má sjá grófa áætlun um vatnsnot á ári [Hagstofan 2002]. Ætla má að í tölum fyrir landbúnað sé fiskeldi langstærsti parturinn og þar sé bæði um að ræða ferskvatn og sjó. Nánari greining gefur til kynna að not fiskeldis í dag (hefur ekki breyst að ráði frá 2002) sé um 32 milljónir m³ á ári eða nærfellt 3 sinnum meira en iðnaður í landinu notar á sama tímabili.

Fyrir utan vatnsmagn sem fiskeldi notar skiptir varmi miklu máli varðandi hraða fiskvaxtar. Sem dæmi er vaxtarhraði bleikju frá 100 grömmum í 1 kg um 500 dagar við 5°C en við 10°C vex sami fiskur úr 100 grömmum í kíló á 300 dögum. Aukinn vaxtarhraði þýðir aukinn veltuhraða og betri nýtingu á eldisrými. Það gefur því auga leið að ef það þarf um 2.500-3.000 sekúndulítra af vatni til að framleiða 1000 tonn af fiski, þarf mikið af heitu vatni til að hita nýtt vatn um hverja gráðu. Ef hita ætti nokkur hundruð sekúndulítra yrði sá kostnaður ofviða flestum ef ekki öllum landeldisstöðum í rekstri í dag.

Það vandamál sem lýst hefur verið hérna í innganginum er nauðsynlegt að leysa til þess að landeldi á ferskvatnsfiski geti orðið að iðnaði á Íslandi. Markmið verkefnisins var að prófa ódýra og einfalda leið til þess að draga úr vatnsnotkun í bleikjueldi. Gert var ráð fyrir því að hægt væri að nýta vatn í bleikjueldi 4-6 falt betur en nú er gert. Með því að nýta vatnið betur mætti auka framleiðslu fiskeldisstöðvanna án þess að aflað sé meira vatns með tilheyrandi kostnaði. Annar kostur við betri nýtingu á eldisvatni er að með því má draga úr sveiflum í hitastigi og hækka eldishita, en hvort tveggja leiðir til betri vaxtar hjá fiskunum.

Markmið og framtíðarsýn

Nauðsynlegt er að landkostir og aðstæður séu hentugar fyrir uppbyggingu fiskeldis. Á síðastliðnum árum hafa jarðvarmavirkjanir sótt á við raforkuframleiðslu. Aukaafurð þessarar raforkuframleiðslu er kælivatn/sjór sem er upphitað lindavatn/sjór af mjög góðum gæðum eftir að hafa verið síað af berglögum í langan tíma. Þessu vatni skilar orkuverið frá sér á bilinu 25-40°C heitu og um umtalsvert magn er að ræða. Sem dæmi má nefna að lítil stöð Orkuveitu Húsavíkur skilar frá sér 250 sekúndulítrum af 25°C heitu lindarvatni og Reykjanesvirkjun Hitaveitu Suðurnesja skilar 3000 sekúndulítrum af 40°C heitu vatni með 23 prómill seltu. Á báðum stöðum er mikið af öðru vatni sem nota mætti til að stilla af óskaða seltu og hita, en þess má geta að lítið hefur verið gert í að nýta þessa aukaafurð til fiskeldis og er því hér um að ræða vannýta auðlind sem minnkar heildarvirði virðiskeðjunnar sem raforkuframleiðsla með jarðvarma samanstendur af.

Tækni við nýtingu vatns í fiskeldi hefur fleygt fram á síðustu árum og hefur verð á eldisbúnaði lækkað. Til að raunhæft sé að nýta jarðhita til eldis verður að vera hægt að endurnýta vatn og varma. Búnaður felur í sér vatnshreinsun og endurnot vatns og varma með sem bestum hætti sem er nauðsynlegt til að hægt sé að byggja stærðarhagkvæmar einingar.

Mikill hluti þess búnaðar sem nýttur var í þessu verkefni er reynd tækni en þó ekki á svo stórum skala sem reyna á hér í bleikjueldi við íslenskar aðstæður. Að auki er sá lífhreinsibúnaður sem prófaður var með búnaði þessa verkefnis uppfinning eins þátttakenda í verkefninu og hefur einkaleyfi verið veitt í Bandaríkjunum en er enn í svokölluðu EPO – umsóknaferli¹ í Evrópu. http://www.esi.info/documents/GEA2 Cross ML.pdf

Hafa ber í huga þá staðreynd áður en lengra er haldið að nauðsynlegt er í hverju endurnýtingarkerfi að geta skipt út 20% af vatni á dag [Timmons, et al. 2001 bls 438]. Í raun er mun einfaldara og ódýrara að skipta út enn meira vatni, eða sem nemur 50-100% á dag. Þar sem viðstöðutími vatns í kerjum er um 30-60 mínútur útheimtir það þó um 20-30 falda endurnýtingu. Ef eldisstöðin er það stór að hagkvæmi stærðar nýtist bæði við framleiðslu fisks og við vinnslu hans er um verulegt magn vatns að ræða sem ekki er á færi margra þjóða að standa undir vegna kostnaðar við varmaorku sem gerir samkeppnisstöðu Íslands góða einmitt á þessu sviði.

Til að útskýra verð á varmaorku er eftirfarandi dæmi. Gera má ráð fyrir að 100.000 kr þurfi til að hita 100 sekúndulítra af vatni um 1°C á mánuði. Sem dæmi má nefna að ef framleiða á 100 tonn af bleikju á ári í hefðbundnu gegnumstreymiskerfi og nota við það sjó sem hita þarf úr 7°C í 14°C, þarf um 7×105 tonn af heitu vatni sem kostar um 20 M krónur á ári. Augljóst er að notkun varmaorku er ekki fjárhagslega hagkvæmur kostur í flestum tilfellum nema vatn sé nýtt að minnsta kosti 10-30 sinnum betur en í hefðbundnum gegnumstreymiskerfum, með endurnýtingu.

Tæknileg markmið

Eitt af markmiðum þessa verkefnis var að sýna fram á að hægt sé að fjórfalda framleiðslugetu bleikjueldisstöðva án þess að afla meira vatns en stöðvar nýta nú þegar. Í einföldu gegnumstreymiskerfi

þarf um 0,7 L kg⁻¹·mín⁻¹. Þær bleikjueldisstöðvar sem nýta vatn best í dag miða við að grunndæling sé 0,2 L·mín⁻¹ ·kg⁻¹. Við teljum raunhæft að minnka þetta enn frekar eða niður í 0,05 L kg⁻¹·mín⁻¹ með einfaldri hreinsun á vatninu. Einnig var í verkefninu kannað hvort hægt væri að ganga ennþá lengra í því að draga úr vatnsþörf í bleikjueldi með notkun nýrrar gerðar lífhreinsis.

Víðast hvar takmarkast hámarksstærð landeldisstöðva endanlega af aðgengi að vatni, þ.e. hversu mikið af köldu vatni er hægt að ná til eldis og jarðhita sem hagkvæmt er að virkja. Rekstarkostnaður eldisstöðva er háður stærð þeirra og almennt gildir að stærri stöðvar eru hagkvæmari í rekstri en minni stöðvar⁹. Því er ávinningur verkefnisins fyrir aukið verðmæti fiskeldis eftirfarandi:

- 1) Stærri og hagkvæmari eldisstöðvar. Með því að bæta nýtingu á vatni má fjórfalda framleiðslu án þess að aflað sé meira af heitu og köldu vatni. Ekki er óraunhæft að áætla að framleiðslukostnaður geti lækkað um 20% við það eitt að auka framleiðslu úr 250 í 1000 tonn á ári. Betri vatnsnot gefa því kost á stækkun einingar á hverjum stað. Stöðvar sem framleiða 500-2000 tonn sem nýta mjög vel hagkvæmi stærðar.
- 2) Lægri framleiðslukostnaður. Með því að bæta vatnsnot má einnig lækka rafmagnskostnað, þar sem orkuþörf hringdælingu úr keri í loftara er mun minni en orkuþörf frumdælingar úr vatnsbóli í loftara. Einnig má nýta heitt vatn margfalt betur og viðhalda þannig stöðugum kjörhita til vaxtar. Orkukostnaður vegna rafmagns og hita getur numið 15-20% af framleiðslukostnaði í stórri eldisstöð. Þar er því nokkuð svigrúm til þess að draga úr framleiðslukostnaði með því að draga úr dælukostnaði og bæta nýtingu á heitu vatni.

Viðskiptaleg markmið

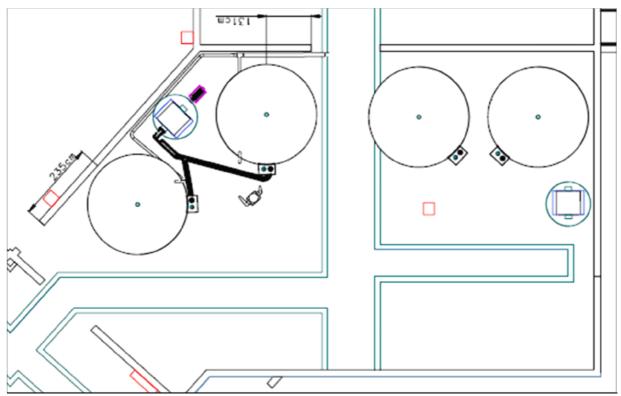
Hólalax ætlar að nýta sér niðurstöður verkefnisins við byggingu nýrrar eldisstöðvar sem framleiða mun 500 tonn af bleikju. Þegar hefur starfsleyfi verið veitt fyrir þessari stækkun og er hún forsenda þess að viðunandi hagkvæmni náist í rekstri.

Mikil eftirspurn er nú eftir bleikju og góð tækifæri til þess að auka framleiðsluna. Niðurstöður verkefnisins gefa eldisstöðvum tækifæri til þess að auka framleiðslu án þess að leggja í kostnað við öflun á köldu og heitu vatni. Gera má ráð fyrir að bleikjueldi muni vaxa hratt hérlendis sökum ýmissa yfirburða sem við höfum gagnvart öðrum þjóðum vegna landgæða og þess að við höfum yfir að ráða mjög góðum kynbættum bleikjustofni.

Matís áætlar að nýta niðurstöður í ráðgjöf til eldisstöðva hérlendis sem erlendis í samvinnu við HolderTimmons Engineering. Matís mun nýta einkaleyfi á lífhreinsi í Evrópu en HolderTimmons Engineering hefur nýtingarrétt í Bandaríkjunum.

2. Aðferðir

Á fyrstu stigum verkefnisins var eldiskerfið hannað og sett upp. Kerfið samanstóð af 4 eldiskerjum ásamt lögnum. Mælisíritum var komið fyrir, alls 14 stk. og þeir tengdir við iðntölvu. Sér dælur voru fyrir hvert kerfi og á þær voru settir hraðabreytar. Tvær tromlusíur voru settar á sín hvor tvö eldiskerfin. Að lokum var sett upp súrefnisframleiðslutæki (oxygen generator) sem hélt súrefnismagni í eldisvökvanum innan réttra marka. Á mynd 1 má sjá afstöðumynd af eldiskerfunum og uppsetningum þeirra í Verinu á Sauðárkróki.

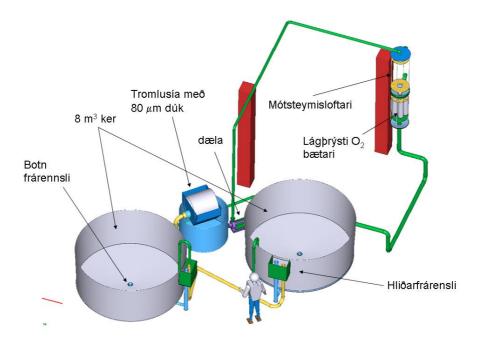


Mynd 1: Yfirlitsmynd yfir eldiskerfi tilraunarinnar

Eldistilraun I

Eftir að eldiskerfið hafði verið sett upp var það prófað og keyrt í nokkurn tíma til að sjá hvernig það virkaði. Niðurstöður þeirrar prófunar voru ásættanlegar og því var hafist handa við *Eldistilraun I*. Fiskar voru aldir í fjórum 8m³ kerjum í tvo mánuði þar sem fiskurinn var látinn vaxa úr 250 gr í 500 gr. Í tveimur kerjanna var vatn endurnýtt (meðal innstreymi 0,008 L·mín⁻¹·kg⁻¹) og í tveimur kerjum var samanburðarhópur sem var alinn í gegnumstreymiskerfi með súrefnisbætingu (meðal innstreymi 0,14 L·mín⁻¹·kg⁻¹). Tilgangur verkþáttarins var kanna hvort hægt væri að ala bleikju með vatnsnotkun 0,01 L·mín⁻¹·kg⁻¹.

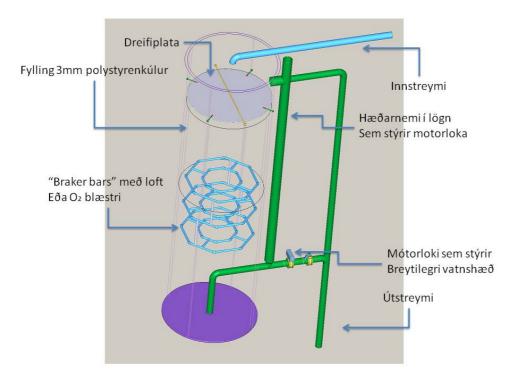
Eftirfarandi, á mynd 2, má sjá þrívíddarteikningu af öðru eldiskerfinu.



Mynd 2 : Kerfi – Orkusparnaður í fiskeldi. Tvö eins kerfi voru smíðuð og var annað notað sem viðmiðunarkerfi. Myndin sýnir þrívíddarteikningu af öðru kerfinu

Eldistilraun II

Í Eldistilraun II var vatnsnotkun færð niður í 0,01 L·mín⁻¹·kg⁻¹ í öðru eldiskerfinu. Í hitt kerfið var settur lífhreinsir. Var það gert til þess að kanna hvort hægt væri að draga enn frekar úr vatnsnotkun í bleikjueldi miðað við það sem tíðkast nú. Lífhreinsir var smíðaður og hann prófaður undir raunverulegu álagi í eldi. Eftirfarandi er skýringarmynd af lífhreinsinum. Tilgangur verkþáttar var að kanna hvort hægt væri að draga enn frekar úr vatnsnotkun með því að hafa lífhreinsi á kerfinu. Þess ber að geta hér að lífhreinsir er nauðsynlegur þegar magn af nýju vatni sem sett er í eldiskerfi fer undir 0,05 L·mín⁻¹·kg⁻¹. Á mynd þrjú má sjá yfirlitsmynd yfir uppbyggingu lífhreinsisins.



Mynd 3 : Yfirlit yfir lífhreinsi. Vatn streymir niður á dreifiplötu og hríslast niður milli plastkúlna í fyllingu. Það sem mótorloki takmarkar flæði út hækkar vatnsborð og fylling hreyfist fram hjá grind með loftflæði sem hrærir til kúlur. Er hámarkshæð er náð opnast mótorloki og vatnshæð fellur uns ferlið er endurtekið. Sjá nánar á www.timmonsaqua.com. Fengist hefur einkaleyfi í Bandaríkjunum

Reglulega voru framkvæmdar lengdarmælingar og þyngdarmælingar á fiskinum í báðum eldistilraununum. Gert var ráð fyrir að það væri gert með mánaðar millibili á hverju stigi tilraunarinnar en ekki reyndist það alltaf mögulegt vegna þess að ekki fékkst til þess tilætlaður mannskapur sem gert hefði verið ráð fyrir í umsókn verkefnisins. Á það sama ekki við þegar kemur að tíðni og fjölda efnamælinga en þær voru framkvæmdar samkvæmt áætlun.

3. Niðurstöður

Eldisrými

Sett voru upp tvö kerfi sem samanstanda hvort um sig af tveimur 8m³ tönkum með tveimur útstreymisopum. Annað útstreymið fyrir miðju á botni kersins og hitt á hlið þess, svokallað "Cornell type" frárennsli. Hægt er að stilla hæð útstreymisops hliðarfrárennslis og þannig stilla af að vissu marki hlutfall þess vatns sem fer um miðjufrárennsli og það sem fer út um hliðarútstreymi. Æskilegt er að geta haft viðstöðutíma vatns um 40 mínútur í keri. Hámarksrennsli í kerfinu er því 400 lítrar á mínútu eða tæplega 7 lítrar á sekúndu.

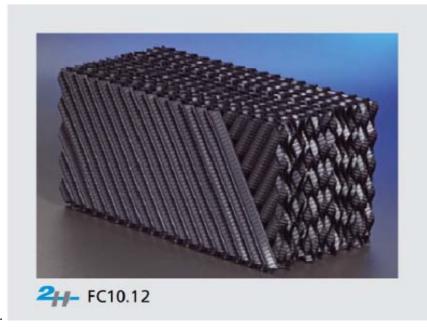
Hugmyndin er sú að hringstreymi í tanki safni gruggi að miðju og það renni síðan burt um það. Hliðarútstreymi á að taka við megninu af vatnsstraumnum og á að innihalda mun minna af gruggögnum. Með því móti má fjarlæga 80% af gruggi með 20% af vatni í gegnum miðjuútstreymi.

Vatnið rennur því næst í svokallaða tromlusíu sem er með $60\mu m$ dúk til að fjarlæga gruggagnir. Dúkurinn er hreinsaður sjálfvirkt með háþrýstisprautun.

Því næst er vatninu dælt í loftara og lágþrýstingssúrefniskerfi.

Loftari og lágþrýstings súrefniskerfi.

Vatni er dælt upp í loftara sem er 0,2m² að flatarmáli. Hámarksflæði á fermetra er því 33 L m⁻² sek⁻¹. Loftarinn inniheldur fyllingu sem dreifir vatninu og sér til þess að sem mest yfirborðsflatarmál myndist og að loft eigi greiðan aðgang upp í gegnum loftarann án þess að mótþrýstingur verði of mikill. Notuð var fylling sem heitir Biodek FB10.12(Mynd 4). Hún er með specific surface area 240 m²/m³. Fyllingin var 0,09m3 að rúmtaki þannig að flatamál sem vatnsborð myndar í fyllingu er 22 fermetrar. Þrýstifall í loftara var mælt við 5,5 sekúndulítra rennsli (27 L m⁻² sek⁻¹) og var lítið og mótstreymi lofts var um 12 rúmtök lofts fyrir hvert rúmtak vatns. Til glöggvunar má sjá fyllinguna sem notuð var í loftarann á mynd



4 hér á eftir.

Mynd 5: Biodek FB10.12 sem notaður var í loftarann

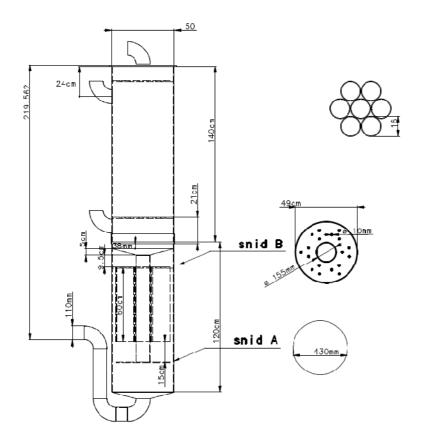
Lágþrýsti-súrefniskerfi: Eftir að vatnið hafði runnið gegnum mótstreymisloftara fer það niður trekt sem sér til þess að fjarlægja allar loftbólur úr vatninu. Því næst myndar það vatnspoll yfir gataplötu sem leiðir vatnið niður um 12 mm göt í 6 hólka sem eru 16 cm í þvermál. Súrefni er leitt í fyrsta hólk og svo koll af kolli milli hólkanna. Vatnshæð yfir gataplötu er stillt af í um 10 – 15 cm vatnshæð. Hægt er að lesa vatnshæð af mæliglasi á hlið loftara. Vatnið fellur um hólka og niður í botntjörn. Dýpt botntjarnar er stillt af með færanlegri botnplötu. Dýpt botntjarnar skiptir máli við að mynda súrefnisloft/vatnsblöndu með sem mestu yfirborðsflatarmáli. Heppilegast er að hafa dýpt á bilinu 20-30cm. Nánar má lesa um virkni lágþrýstingsloftara í eftirfarandi greinum:

Aquacultural Engineering Volume 24, Issue 4, April 2001, blaðsíður 257-277

Aquacultural Engineering Volume 24, Issue 4, April 2001, blaðsíður 245-256).

Að loftun og súrefnisbætingu lokinni er vatninu dælt aftur í eldiskerin um innstreymisrör. Ástæða seinni dælingar er sú að lofthæð í Verinu er ekki nógu há til að fall úr loftara verði nógu mikil til að ná upp nægum vatnsstraum. Afl beggja dæla í kerfinu, bæði að lofturum og undan þeim er stjórnað með hraðabreytum.

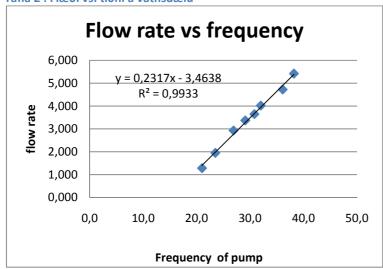
Til nánari glöggvunar má sjá mynd 5 sem sýnir grunnteikningu loftarans sem hann var smíðaður eftir.



Mynd 6: Grunnteikning loftarans

Kvörðun á búnaði: Gerðar voru mælingar á flæði í kerfinu miðað við mismunandi tíðni dælu. Með því er hægt að finna hlutfall milli tíðni dælu og flæðis og nota síðan tíðnitölu til að ákvarða flæðið.





Mælingar voru framkvæmdar með tímamælingum á fyllingu á 20 L fötu sem var vegin að tímamælingu lokinni. Gerðar voru 10 mælingar við hvert flæði af tveimur mælimönnum. Nákvæmni var um 2% og hittni milli mælimanna var einnig um 2%.

Hámarksrennsli sem hægt var að keyra í gegnum kerfið reyndist vera 5,5 L/sek eða 0,33 m³/mín. Viðstöðutími í kerum er því rétt tæpar 50 mínútur eða aðeins hærra en óskandi hefði verði. (40 mínútur). Flöskuhálsinn reynist vera afkastageta loftbólutrektar á milli loftara og lágþrýstisúrefnisbæti. Ekki reyndist gott að laga þann galla nema með nokkrum tilfæringum og var ákveðið að halda áfram með fyrstu tilraun.

Vaxtarmælingar

Eldistilraun I - endurnýting án lífhreinsis

Tilraunin var keyrð dagana 11. apríl til 9. júní 2007. Vaxtarniðurstöður tilraunarinnar má sjá í töflum 3-4 og 6-7. Í töflum 5 og 8 má sjá yfirlit yfir L Kg⁻¹mín⁻¹ og meðaltals L Kg⁻¹ mín⁻¹ í kerfunum.

Kerfi I - Aukin endurnýting eldisvökva

Ker I	Tafla 3 : Vöxtur keri I í Vaxtartilraun I				
		Meðalþyngd í Heildarþungi			
Dagsetning	Fjöldi fiska	gr.	(kg)		
Upphafsfjöldi	1000	260	260,00		
11.apr	998	260	259,48		
24.apr	998	335	334,33		
26.maí	997	460	458,62		
7.jún	996	446	444,22		
Dauði	4	fiskar			

Í töflu 3 hér að ofan má sjá vöxt í keri I í Vaxtartilraun I. Fiskurinn í tilrauninni óx um 186 gr. að meðaltali á þeim tveimur mánuðum sem tilraunin stóð. Ekki gætti mikils dauða í kerinu.

Ker II	Tafla 4 : Vöxt	Tafla 4 : Vöxtur í keri II í Vaxtartilraun I			
Dagsetning	Fjöldi fiska	Meðalþungi (gr)	Heildarþungi (kg)		
Initial	1120	257	287,84		
11.apr	1117	257	287,07		
24.apr	1116	289	322,52		
26.maí	1116	399	445,28		
7.jún	1115	484	539,66		
Mortality	5	fiskar			

Í töflu 4 hér að ofan má sjá vöxt í keri II í Vaxtartilraun I. Fiskurinn í tilrauninni óx um 231 gr. að meðaltali á þeim tveimur mánuðum sem tilraunin stóð. Ekki gætti mikils dauða í kerinu. Að auka ber að merkja að heldur meiri fjöldi fiska var í keri II en í keri I.

Í töflu 5 hér fyrir neðan má sjá yfirlit yfir magn af nýju vatni sem dælt var í kerfið á hverjum tíma. Vatnsrennsli var aukið eftir því sem fiskurinn í kerfinu stækkaði.

	Heild fyrir kefi I				
Heildar	Nýtt vatn	Nýtt vatn	Viðstöðutími		
lífmassi		á kg fisk	vatns		
kg	L min ⁻¹	L min ⁻¹ kg ⁻¹	klukkustundir		
547,8	48,0	0,088	05:33		
546,5	48,0	0,088	05:33		
656,9	55,8	0,085	04:46		
903,9	67,2	0,074	04:00		
983,9	67,2	0,068	04:00		

Tafla 5 : Lífmassi og nýtt vatn inn í kerfi I

Kerfi II - Viðmiðunarkerfi

Ker III	Tafla 6 : Vöxtur í keri III í Vaxtartilraun I			
Dagsetning	Fjöldi fiska	Meðalvigt (gr)	Meðalvigt (kg)	
Upphafsþyngd	1004	259	260,04	
11.apr	1001	259	259,26	
23.apr	1000	335	335,00	
26.maí	1000	447	447,00	
8.jún	1000	479	479,00	
Skráður dauði	4			

Í töflu 6 má sjá vöxt í keri III í Vaxtartilraun I. Fiskurinn í tilrauninni óx um 186 gr. að meðaltali á þeim tveimur mánuðum sem tilraunin stóð. Ekki gætti mikils dauða í kerinu.

Ker IV	Tafla 7 : Vöxtur í keri IV í Vaxtartilraun I			
Dagsetning	Fjöldi fiska	Meðalvigt (gr)	Meðalvigt (kg)	
Initial	1026	253	259,58	
11.apr	1021	253	258,31	
23.apr	1021	310	316,51	
26.maí	1019	375	382,13	
5.jún	1019	394	401,49	
Skráður dauði	7			

Í töflu 7 hér að ofan má sjá vöxt í keri IV í Vaxtartilraun I. Fiskurinn í tilrauninni óx um 231 gr. að meðaltali á þeim tveimur mánuðum sem tilraunin stóð. Ekki gætti mikils dauða í kerinu.

Í töflu 8 hér á eftir má sjá yfirlit yfir magn af nýju vatni sem dælt var í kerfið á hverjum tíma. Vatnsrennsli var aukið eftir því sem fiskurinn í kerfinu stækkaði.

	Heild fyrir kerfi II			
Heildar	Nýtt vatn	Nýtt vatn	Viðstöðutími	
lífmassi		á kg fisk	vatns	
kg	L min ⁻¹	L min ⁻¹ kg ⁻¹	klukkustundir	
519,6	70,2	0,135	03:47	
517,6	67,2	0,130	03:58	
651,5	84,0	0,129	03:10	
829,1	105,0	0,127	02:32	
880,5	160,2	0,182	01:40	

Tafla 8 : Lífmassi og nýtt vatn inn í kerfi II

Niðurstöður varðandi vöxt og viðgang fisks:

Gerðar voru mælingar á vexti fiska með mæliramma frá VAKA fjórum sinnum yfir tímabilið. Svo slysalega vildi til að bilun varð í búnaði og viðvörunarkerfi brást einnig þannig að liðlega 1600 fiskar dóu í kerfi 1 við lok tilraunar. Því var gerð mæling á meðalþyngd þess fisks sem dó til að bera saman við mælingar sem gerðar voru með VAKA ramma. Mælingar gáfu til kynna nokkuð hærri meðalþyngd en Vakaramminn gaf. Skýring getur verið sú að frekar hafi dáið stærri fiskur þar sem hann er frekari á súrefni. Í öllum útreikningum er stuðst við þyngdarmælingar úr Vakaramma.

Mælitímabil 8.4.2007-9.6.2007

	Ke	rfi I	Ker	fi II
	ker 1	ker 2	ker 3	ker 4
Fjöldi fiska (stk)	817	831	1004	1028
Heildarþungi (kg)	414,4	381,4	480,9	405,0
Vigtuðu meðalþyngd (gr)	507,22	458,97		
Vakarammi mþ (gr)	446	484	479	394
Heildarfóðrun á tímabilinu	183,2	188,63	185,1	182
Fóðurtími, dagar	61	61	61	61
%meðal dagvöxtur	1,00	1,04	1,01	0,73
Vöxtur	216	168	221	145
Fóður	140	140	185	182
Fóðurnýting	0,85	0,74	0,84	1,25

Tafla 9: Mælingar á vexti og viðgangi fisk í endurnýtingarkerfi (kerfi I) og viðmiðunarkerfi (kerfi II).

Rétt er að nefna það að Kerfi II viðmiðunarkerfi er í raun einnig endurnýtingarkerfi en með minni endurnýtingu. Ekki eru vatnslagnir nógu sverar í húsnæði Versins þar sem tilraunin var framkvæmd til að hafa viðmiðunarkerfi hreint gegnumstreymiskerfi.

Helstu niðurstöður eru þær að vöxtur fiska var svipaður í öllum kerum en þó sýnu lakastur í keri 4 í viðmiðunarkerfi, Kerfi II. Ástæðan er talin vera sú að rennihurð var nálægt því keri sem olli nokkru stressi þegar hún var opnuð – sem gerðist nokkuð oft á dag. Því var klætt af milli hurðar og kers í áframhaldandi tilraunum.

Vöxtur var í raun um 20-30% betri en vaxtatöflur fyrir bleikju segja til um samkvæmt mælingum Evu Brännäs *et al.* á bleikju úr sænska kynbótaverkefninu. Þar kom fram að vöxtur bleikju fylgir jöfnunni (STP)=[(0,722+0,174 T)]-(0,09+0,019 T)*In W, þar sem T er hiti í °C og W = þyngd fisks í grömmum. STP stendur fyrir specific growth rate.

Ástæða þess er eflaust að mestu sú að fiskurinn hafði komið úr verri aðstæðum, hafði verið bólusettur og sveltur nokkuð fyrir tilraun og því átt inni vöxt sem hann hefur tekið út við góðar aðstæður í tilraun.

Fóðurnýting reyndist einnig mjög góð eða á bilinu 0,74 – 0,85 nema fyrir ker 4 sem eflaust stafar af því stressi sem fyrr er lýst.

Allmennt má segja að enginn munur virðist vera á vexti og þrifum fisks við þessa endurnýtingu eða allt að um 0,070 L min⁻¹ kg⁻¹.

Mælingar á efnaþáttum vatns:

Fylgst var með þeim efnaþáttum sem mest skipta máli fyrir viðgang fisks en þeir eru styrkur súrefnis, styrkur CO_2 , styrkur gruggs og styrkur ammóníaks í vatninu.

	kerfi I		Ker	fi II
	ker 1	ker 2	ker 3	ker 4
Meðal súrefnisgildi í keri:	7,55	7,85	7,76	8,56

Tafla 10: meðalstyrkur súrefnis í kerum - 40 daga meðaltal

Súrefni reynist vera mjög svipað í öllum kerum nema því fjórða í viðmiðunarkerfi (Kerfi II). Súrefnisgildi var rétt tæplega 8 mg/L . Æskilegt hefði verið að hafa það eitthvað hærra en til þess hefði þurft að auka vatnsskipti endurnýtingar í keri um 10-15%, en það var ekki mögulegt vegna fyrrnefnds hönnunargalla.

 CO_2 var mælt daglega og reyndist að jafnaði nokkuð lágt og virtist búnaður vel ráða við að fjarlægja hann úr vatninu.

Koltvísýring er einfaldast að mæla óbeint út frá pH mælingu auk mælingar á heildarkarbónatstyrk vatnsins. Til að svo megi verða, verða einnig pKa₁ og pKa₂ fyrir karbónat að vera þekkt við það hita- og seltustig sem um er að ræða. Í raun nægir að þekkja pKa₁ þar sem pKa₂ veldur mjög litlum breytingum á því sýrustigsbili sem um ræðir. Til að reikna út pKa1 má nota jöfnuna:

$$pK_1 = \frac{3404,7}{T} + 0,03278 \times T - 0,1917(S/1,806)^{1/3} - 14,712$$

þar sem T er hitastig í °K og S er selta í prómill (‰). Kolsýru í vatni má síðan reikna út frá:

$$[H_2CO_3^*] = C_{tot} \times 10^{(pK_1 - pH)}$$

Styrkur þess var að jafnaði undir 10 mg/L sem er viðunandi en óx nokkuð síðustu daga tilraunar. Ástæða er sú sama og fyrir aðeins of lágum súrefnisstyrk, bæta hefði þurft vatnsskipti og ná viðstöðutíma niður í 35-40 mínútur.

Grugg var mjög svipað allan tímann í öllum kerum eða milli 20-30 mg/L það er viðunandi. Grugg má minnka frekar með örari vatnskiptum.

Ammoníak:

Pau ólífrænu nitursambönd sem mestu máli skipta í fiskeldi eru ammóníak (NH3), ammóníum (NH $^+$ 4), nítrat (NO $_3$ $^-$) og nítrít (NO $_2$ $^-$). Útskilnaður nitursambanda hjá beinfiskum er 60%-90% á formi NH3 og NH+4, en einnig eru nitursamböndin skilin út á formi þvagefna og sem önnur lífræn nitursambönd. Hlutfall þvagefnis í heildar niturútskilnaði er nokkuð hærri hjá sjávarfiskum (30-40%) en hjá ferskvatnsfiskum (Jobling 1994). Útskilnaður NH3 og NH+4 á sér stað í gegnum tálknin en önnur nitursamböndin eru skilin út með þvagi og í gegnum húð.

Samanlagður styrkur köfnunarefnis á báðum formum er nefndur heildarstyrkur ammóníaks. Heildarstyrkur ammoníaks er táknað sem NH4-N skammstafað á ensku sem TAN (Total Ammonium Nitrogen). Það hlutfall af heildarstyrk ammóníaks sem er á formunum NH₄⁺ og NH₃ ræðst af pH, hita og

seltu. Það er NH_3 sem er sérlega hættulegt fiski, það er fituleysanlegt og getur því auðveldlega farið í gegnum frumuhimnu. Hár styrkur af ammoníaki getur dregið úr vexti fiska og aukið næmni þeirra fyrir sjúkdómum. Of hár styrkur af ammóníaki getur leitt til dauða. "European Inland Fisheries Advisory Commission" (EIFAC 1970) hafa sett viðmiðunarmörk um að styrkur NH_3 skuli ekki fara yfir 0,02 mg L^{-1} . Sá styrkur NH_3 sem er almennt talinn banvænn fyrir laxfiska (salmonids) er 0,83 til 1,1 mg L^{-1} sé þessum styrk viðhaldið í 4 daga og 0,14 til 4,6 mg L^{-1} fyrir aðra beinfiska (nonsalmonids) (Flis 1968; Chiba 1980).

Ammoníak var mælt með svokölluðu fenól-hypoklór hvarfi. Heildarstyrkur ammoníumjóna var ávallt undir 1 mg í lítra. Við þau skilyrði er styrkur ójónað ammoníaks á forminu NH₃ vel undir hættumörkum. Því er ljóst að styrkur ammoníaks verður ekki hamlandi við endurnýtingu að um 0,05 mg L⁻¹ kg⁻¹.

Eldistilraun II

Eftir stóð að reyna frekari vatnsspörun. Annars vegar var prófuð virkni lífhreinsa við kerfið og hins vegar meiri vatnsspörun með túpusetkeri.

Prófanir með virkni lífhreinsa var sett upp sem nemendaverkefni við UNU háskólann (Fisheries Training Program) og voru framkvæmdar af Mercedes Isla Molleda frá Centro de Investigaciones Pesqueras á Kúbu. Sett voru upp tvö minni kerfi með tveimur 700 lítra kerum hvort. Annað var með lífhreinsi sem samsvarar HTE- lífhreini en er nokkuð einfaldara að gerð. Ekki var unnt að nota tromlusíur við þessi kerfi þar sem ekki var til nógu lítil tromlusía fyrir þau. Var brugðið á það ráð að nota svokallaðar sandsíur í staðinn. Var það nokkur galli því þær þarf að bakspúla að minnsta kosti einu sinni á dag en nokkur vanhöld voru á því í tilrauninni sem kom niður á gildi rannsókna. Þó má segja að megin niðurstaða sé sú að Sequenser lífhreinsir virðist virka ágætlega fyrir bleikjueldi og er vel nýtilegur til að spara vatn enn frekar. Skýrsla úr þessu verkefni fylgir með sem viðauki I.

Fengist hefur einkaleyfi á HTE-Lífhreinsi í Bandaríkjunum og á sama tíma hafa Bandarískir samstarfaðilar gert prófanir í laxeldi í BNA. Þeir hafa meðal annars verið settir upp í DOMSEA farms í Washington. Hægt er að skoða myndir af uppsetningu á stórum filter á síðunni www.aquacare.com/video.htm.

Eldistilraun II var einnig sett upp sem nemendaverkefni og var framkvæmd af Hans van Someren Gréve og Tom Martens frá University of applied sciences Has Den Bosch í Hollandi.

Þar var reynd frekari vatnsspörun og einnig var prófað að nota svokallaðan tubesettler inn í kerfið.

Hann reyndist með afbrigðum illa og sýndi í raun fram á mikilvægi þess að ná öllu gruggi sem skjótast úr kerfinu. Niðurstöður voru birtar í lokaskýrslu þeirra sem er hér aftanvið sem Viðauki II.

4. Umræður

Niðurstaða þessarar rannsóknar er sú að hægt er að framleiða í kringum sjö sinnum meira af lífmassa í fiskeldi á landi með því vatnsmagni sem notað er í dag. Til þess að það sé hægt þarf að hafa eftirfarandi atriði í huga:

- Mjög mikilvægt er að losa grugg sem fyrst úr vatninu því það getur orðið valdur að miklum vandræðum líkt og kom upp í einu skrefi í þessari rannsókn. Því ber að leita allra leiða til að losa grugg úr endurnýtingarkerfinu eigi það að skila ætluðum tilgangi og því er tromlusía nauðsynlegur búnaður og ber að sía allt vatnið við hvern hring endurnýtingar. Í síunni ætti að vera 100 µm dúkur en hann hreinsar allar agnir sem minnkað geta virkni eldiskerfisins.
- Nægur straumur verður að vera í eldiskerjunum og æskilegt er að vatnskiptahraði sé ekki minni en 45 mínútur til að tryggja sjálfhreinsun og að fullnægja súrefnisþörf fiska við mikla þéttni.
- Lágþrýstisúrefnismettari (LHO) gefur ágæta raun við mettun súrefnis í kerfi. Í stórum djúpum kerum má eflaust einnig nota nýjar gerðir af fínúða loftunarsteinum (loftbólustærð 100 - 500 μm). Þeir virka vel á miklu dýpi og sýnt góða nýtni. Þeir þurfa allmikinn þrýsting á súrefni eða milli 1,5-3 bör.
- Mótstreymisloftari af þeirri gerð sem reyndur var er nægjanlegur til að halda styrk CO2 innan viðunandi marka. Þó er sú gerð sem notuð var af fyllingu, FC10.12, með fullþröng hólf. Réttara væri að nota fyllingu sem hefur meira bil milli platna og er mælt með gerðinni FC10.27.

Lífhreinsir er nauðsynlegur útbúnaður þegar endurnýting er meiri en 0,03-0,05 L kg min. Hann losar ammoníak úr eldisvökvanum. Lífhreinsirinn sem notaður var í þessari rannsókn hefur sýnt sig að virka vel og einkaleyfi hefur fengist á hönnun hans.

5. Þakkir

Höfundar þakka Tækniþróunarsjóð fyrir veittan stuðning

6. Viðaukar

Viðauki 1

Mercedes Isla Molleda. Water quality in recirculating aquaculture systems for Arctic Charr (*Salvelinus alpinus* L.) culture.

Viðauki 2

Hans van Someren Gréve, Tom Martens. Research on growth of Arctic Charr and water quality in water re-use systems at different water exchange rates.



WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEMS FOR ARCTIC CHARR (Salvelinus alpinus L.) CULTURE

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ABSTRACT

Recirculating aquaculture systems (RAS) for fish culture have been used for more than three decades. The interest in RAS is due to their advantages such as greatly reduced land and water requirements in places where water resources are limited; but RAS also have disadvantages like the deterioration of the water quality if the water treatment processes within the system are not controlled properly. The water quality problems in RAS are associated with low dissolved oxygen (DO) and high fish waste metabolite levels in the culture water. The objective of this study is to compare water quality in a RAS with water quality in a limited reuse system (LRS) for Arctic charr culture taking into account the oxygen demands of the fish, the metabolites production by the fish, the removal of CO₂ by the aerators, the removal of ammonia by the biofilter and the removal of waste products in the reused water. The experiment was conducted in Verid, the Aquaculture Research Facilities of Holar University College, Iceland, during 4 weeks. The two different systems were compared during the experiment: a RAS with a biofilter and a LRS. The results of this study showed that the water quality parameters in both systems were well within the acceptable levels for Arctic charr culture and the water quality was better in the LRS than in the RAS; the important role of the biofilter unit in the RAS was demonstrated and the necessity to control all the water treatment processes within the system, especially when the RAS is using sand filters as one of the water treatment components of the system.

Keywords: Arctic charr, water quality, recirculating aquaculture systems, fish culture.

TABLE OF CONTENTS

1	INTRODUCTION	5
	1.1 Cuba: current situation	6
2	LITERATURE REVIEW	8
	2.1 WATER QUALITY IN RECIRCULATION AQUACULTURE SYSTEMS (RAS)	
	2.1.1 Dissolved oxygen (DO) and carbon dioxide (CO ₂) levels	8
	2.1.2 Oxygen consumption (MO ₂)	11
	2.1.3 Nitrogen metabolites levels	11
	2.1.3.1 Ammonia levels	
	2.1.3.2 Nitrite (NO ₂ -N) and nitrate (NO ₃ -N) levels	
	2.1.4 pH levels, the relationship with nitrogen and inorganic carbon metabolites production	
	recirculation systems	
	2.1.5 Solids concentration levels	
	2.2 ARCTIC CHARR AS A FARMING SPECIES IN ICELAND	15
3	MATERIALS AND METHODS	17
4	RESULTS	20
	4.1 DISSOLVED OXYGEN (DO) LEVELS AND OXYGEN CONSUMPTION (MO ₂) IN THE SYSTEMS	20
	4.2 PH WATER LEVELS IN THE SYSTEMS	
	4.3 TOTAL INORGANIC CARBON (TIC) AND CARBON DIOXIDE (CO ₂) LEVELS IN THE SYSTEMS:	20
	REMOVAL RATE OF CARBON DIOXIDE (CO_2).	22
	4.4 NITROGEN METABOLITES	
	4.4.1 Total ammonia nitrogen (TAN) concentrations and removal rate of TAN in the system	
	4.4.2 Unionised ammonia (NH ₃ -N)	
	4.4.3 Nitrogen metabolites	26
	4.5 TOTAL SUSPENDED SOLIDS (TSS) LEVELS AND REMOVAL RATE OF TSS IN THE SYSTEMS	27
5	DISCUSSION	29
	5.1 DISSOLVED OXYGEN (DO) LEVELS AND OXYGEN CONSUMPTION (MO_2) IN THE SYSTEMS	29
	5.2 PH LEVELS IN THE SYSTEMS	29
	5.3 TOTAL INORGANIC CARBON (TIC) LEVELS AND CARBON DIOXIDE (CO_2) LEVELS IN THE	
	SYSTEMS: REMOVAL RATE OF CARBON DIOXIDE (CO ₂)	30
	5.4 Total ammonia nitrogen (TAN) and unionised ammonia (NH $_3$) levels in the	
	SYSTEMS: REMOVAL RATE OF TAN	
	5.5 BIOFILTER PERFORMANCE IN THE RAS	
	5.6 TOTAL SUSPENDED SOLID (TSS) LEVELS IN THE SYSTEMS: REMOVAL RATE OF TSS	32
6	CONCLUSIONS	33
A	ACKNOWLEDGEMENTS	34
R	REFERENCE LIST	35
A	APPENDIX: TABLES OF MEASUREMENTS	39

LIST OF FIGURES

Figure 1: Effects of pH on the relative proportions of total CO ₂ , HCO ₃ , and CO ₃ ² . The mole
fraction of a component is its decimal fraction of all the moles present (Boyd 2000)9
Figure 2: Typical startup curve for a biological filter showing time delays in establishing
bacteria in biofilters (Timmons et al. 2002).
Figure 3: Aquaculture systems used for the experiment. Limited reuse system (LRS) and
recirculating aquaculture system (RAS) with biofilter17
Figure 4: General diagram of the systems and measurement points. Recirculating aquaculture
system (RAS) with biological filter coupling and limited reuse system (LRS) without
biological filter, where (1) inlet water after total treatment, (2) fish culture tank 1, (3) fish
culture tank 2, (4) inlet new water and (5) outlet water from BF19
Figure 5: Dissolved oxygen (DO) concentrations (mg L ⁻¹) in the water inlet tanks and in the
outlet water from the tanks and the oxygen consumption rate (MO ₂) of the fishes (mg O ₂ min ⁻¹
kg ⁻¹) in each system during the experimental time
Figure 6: pH levels in the tanks water, in the water inlet tanks and in the new inlet water to the
system for each system during the experimental time
Figure 7: Total inorganic carbon (TIC) concentrations (mg L ⁻¹) in the outlet and inlet water
tanks and in the new inlet water to the system for each system during the experimental time.
Figure 8: Carbon dioxide (CO ₂) concentrations (mg L ⁻¹) in the outlet water from the tanks and
in the inlet water tanks and CO ₂ removal rate from the system (mgCO ₂ min ⁻¹ kg ⁻¹) for each
system during the experimental time23
Figure 9: Total ammonia nitrogen (TAN) concentrations (mg L ⁻¹) in the outlet water from the
tanks and in the inlet water tanks and TAN removal rate (mg TAN min-1 kg-1) for each system
during the experimental time24
Figure 10: TAN concentration levels in different water points in the RAS at days 15 and 18 of
the experimental period and at day 26, one week after the end of the experiment, before and
after 5 hours to clean the sand filter.
Figure 11: Unionised ammonia (NH ₃ -N) concentrations (mg L ⁻¹) for each system in the outlet
water from the tanks and in the water inlet tanks and in the outlet water from the biofilter in
the RAS, during the experimental time. The red line in both charts indicates the unionised
ammonia (NH ₃ -N) concentrations limit of water quality (mg L ⁻¹) for salmonids culture26
Figure 12: Nitrogen metabolites (TAN, NO ₂ -N and NO ₃ -N) concentrations (mg L ⁻¹) in the
outlet water from the biofilter in the RAS.
Figure 13: Total ammonia nitrogen (TAN) concentrations (mg L ⁻¹) in the outlet water from
the tanks and in the inlet water tanks for the RAS during three stages at the same experimental
day (18), where NC (normal conditions), A 30 min TF (after 30 minutes of turn off the
biofilter) and A 1 h TF (after 1 hour of turn off the biofilter).
Figure 14: Total suspended solids (TSS) concentrations (mg L ⁻¹) in the outlet water from the
tanks and in the inlet water tanks for each system (LRS and RAS) during the experimental
time
Figure 15: Total suspended solids (TSS) removal rate (%) for LRS and RAS during the
experimental time

LIST OF TABLES

Table 1: Lethal levels of NH ₃ -N (concentration of nitrogen bound as NH ₃) for some	
aquaculture species.	12
Table 2: Daily measurements in the LRS tank No. 1 between days 0 – 9	39
Table 3: Daily measurements in the LRS tank No. 1 between days 10 – 19	40
Table 4: Daily measurements in the LRS tank No. 2 between days 0 – 9	41
Table 5: Daily measurements in the LRS tank No. 2 between days 10 – 19	42
Table 6: Daily measurements in the new water inlet to LRS between days $0-9$	43
Table 7: Daily measurements in the new water inlet to LRS between days 10 – 19	43
Table 8: Values of different water quality parameters calculated in LRS tank No. 1 two tin	nes
per week during the experimental time and their Removal rate values	44
Table 9: Values of different water quality parameters calculated in LRS tank No. 2 two tin	nes
per week during the experimental time and their Removal rate values	44
Table 10: Values of different water quality parameters calculated in the water inlet tanks of	of
the LRS two times per week during the experimental time and the water flow using inside	the
tanks in the system	45
Table 11: Values of different water quality parameters calculated in the new water inlet to	1
LRS two times per week during the experimental time and the water flow using within the	•
system	45
Table 12: Daily measurements in the RAS tank No. 1 between days $0-9$	47
Table 13: Daily measurements in the RAS tank No. 1 between days 10 – 19.	48
Table 14: Daily measurements in the RAS tank No. 2 between days $0-9$	49
Table 15: Daily measurements in the RAS tank No. 2 between days 10 – 19	50
Table 16: Daily measurements in the new water inlet to the RAS between days $0-9$	51
Table 17: Daily measurements in the new water inlet to the RAS between days $10 - 19$	51
Table 18: Daily measurements in the outlet water from the biofilter in the RAS between da	ays
3 – 12	52
Table 19: Daily measurements in the outlet water from the biofilter in the RAS between da	ays
13 – 19.	52
Table 20: Values of different water quality parameters calculated in RAS tank No. 1 two	
times per week during the experimental time and their Removal rate values	53
Table 21: Values of different water quality parameters calculated in RAS tank No. 2 two	
times per week during the experimental time and their Removal rate values	53
Table 22: Values of different water quality parameters calculated in the water inlet tanks of	of
the RAS two times per week during the experimental time.	
Table 23: Values of different water quality parameters calculated in the new water inlet to	
RAS two times per week during the experimental time.	
Table 24: Values of different water quality parameters calculated in the outlet water from	
biofilter in the RAS two times per week during the experimental time	54

1 INTRODUCTION

Recirculating aquaculture systems (RAS) consist of an organised set of complementary processes that allow at least a portion of the water leaving a fish culture tank to be reconditioned and then reused in the same fish culture tank or other fish culture tanks (Timmons *et al.* 2002).

Recirculating systems for holding and growing fish have been used by fisheries researchers for more than three decades. Attempts to advance these systems to commercial scale food fish production have increased dramatically in the last decade although few large systems are in operation. The renewed interest in recirculating systems is due to their perceived advantages such as greatly reduced land and water requirements; reduced production costs by retaining energy if the culture species require the maintenance of a specific water temperature, and the feasibility of locating production in close proximity to prime markets (Dunning *et al.* 1998).

However, the RAS also have disadvantages. The most important is the deterioration of the water quality if the water treatment process within the system is not controlled properly. This can cause negative effects on fish growth, increase the risk of infectious disease, increase fish stress, and other problems associated with water quality that result in the deterioration of fish health and consequently loss of production (Timmons *et al.* 2002). The water quality in RAS depends on different factors most importantly the source, the level of recirculation, the species being cultured and the waste water treatment process within the system (Sanni and Forsberg 1996, Losordo *et al.* 1999).

Most water quality problems experienced in RAS were associated with low dissolved oxygen and high fish waste metabolite concentrations in the culture water (Sanni and Forsberg 1996). Waste metabolites production of concern include total ammonia nitrogen (TAN), unionised ammonia (NH₃-N), nitrite (NO₂-N), nitrate (NO₃-N) (to a lesser extent), dissolved carbon dioxide (CO₂), suspended solids (SS), and non-biodegradable organic matter. Of these waste metabolites, fish produce roughly 1.0-1.4 mg L⁻¹ TAN, 13-14 mg L⁻¹ CO₂, and 10-20 mg L⁻¹ TSS for every 10 mg L⁻¹ of DO that they consume (Hagopian and Riley 1998). However, maintaining good water quality conditions is of primary importance in any type of aquaculture system, especially in RAS.

Prospective users of aquaculture systems need to know about the required water treatment processes to control temperature, dissolved gases (oxygen, carbon dioxide, and nitrogen), pH, pathogens, and fish metabolites such as solids (both dissolved and particulate) and dissolved nitrogen compounds (ammonia, nitrite and nitrate) levels in the culture water; the components available for each process and the technology behind each component (Losordo *et al.* 1999).

Water reuse systems generally require at least one or more of the following treatment processes, depending upon their water-use intensity and species-specific water quality requirements (Losordo *et al.* 1999):

• Sedimentation units, granular filters, or mechanical filters to remove particulate solids.

- Biological filters to remove ammonia.
- Strippers/aerators to add dissolved oxygen and decrease dissolved carbon dioxide or nitrogen gas to levels closer to atmospheric saturation.
- Oxygenation units to increase dissolved oxygen concentrations above atmospheric saturation levels.
- Advanced oxidation units (i.e. UV filters or units to add ozone) to disinfect, oxidise organic wastes and nitrite, or supplement the effectiveness of other water treatment units.
- pH controllers to add alkaline chemicals for maintaining water buffering or reducing dissolved carbon dioxide levels.
- Heaters or chillers to bring the water temperature to a desired level.

A key to successful RAS is the use of cost-effective water treatment system components. Water treatment components must be designed to eliminate the adverse effects of waste products (Losordo *et al.* 1998). In recirculating tank systems, proper water quality is maintained by pumping tank water through special filtration and aeration and/or oxygenation equipment. Each component must be designed to work in conjunction with other components of the system. To provide a suitable environment for intensive fish production, recirculating systems must maintain uniform flow rates (water and air/oxygen), fixed water levels, and uninterrupted operation (Masser *et al.* 1999).

Currently, freshwater recirculating systems are used to raise high value species or species that can be effectively niche marketed, such as Salmon smolt and ornamental fishes, as well as fingerling and food-sized tilapia, hybrid-striped bass, yellow perch, eels, rainbow trout, African catfish, Channel catfish, and Arctic charr, to name just a few. Additionally, saltwater reuse systems are being used to produce many species at both fingerling and food-size, including flounder, sea bass, turbot, and halibut; water reuse systems are also used to maintain many kinds of coldwater and warm water brood stock fish (Summerfelt *et al.* 2004a).

1.1 Cuba: current situation

Aquaculture in Cuba has been developed as commercial activity since 1976, mainly with the culture of different fresh water species such as tilapia (*Oreochromis spp.*), silver carp (*Hypophthalmichthys molitrix*), Channel catfish (*Ictalurus punctatus*) and tenca (*Tinga tinga*) in dam rivers as extensive culture. The year 1986, was the beginning of the marine species culture development with the culture of white shrimp (*Litopenaeus schmitti*) in land ponds as semi intensive culture with a total production of 27 tons that year (Cuban Statistic Annual Fisheries 2004).

Currently, white shrimp culture production in Cuba is the second line of exportation income from the Ministry of Fishing Industry to the country's economy with approximately 1700-2000 tons of total production per year, 2400 tons in 2006 after the introduction of the Pacific white shrimp (*Litopenaeus vannamei*) in 2004 to use this specie for the culture, in approximately 2300 hectares of land culture ponds (Cuban Statistic Annual Fisheries 2006). On the other side, the total fresh water aquaculture production during this decade was around 32,000-43,000 tons, and the main species were silver carps, with 12,300-25,600 tons production per year, tenca

between 13,700-15,000 tons per year and tilapia between 4500-5000 tons per year (Cuban Statistic Annual Fisheries 2006). The fresh water aquaculture production is used to supply local market demand and some tourist places on the island such as restaurants and hotels.

The Cuban marine fish culture production is low. One of the major experiments in marine fish culture in the country was conducted from 1999 until 2001 with the introduction of juveniles of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) to culture in net cages at the open sea for commercial business in four parts of the island shelf (Isla *et al.* 2006).

At present, Cuba has three experimental hatcheries for marine fish culture, one of them, the oldest one with more than ten years building, to produce mutton snapper (*Lutjanus analis*) and common snook (*Centropomus undecimales*), located in Camaguey province, at the south central part of the country; and the other two, to produce cobia (*Rachicentron canadum*), one of them located in Cienfuegos province, at the southeast part and the other in Granma province, at the southwest part of the country, with around 2 and 7 years building, respectively. At present, these hatcheries are used to maintain the brood stocks of these species in flow-through aquaculture systems.

There are no RAS in use in Cuba today, but the structure and design of the hatcheries permit installation of RAS to improve operation with a consequent reduction in the water used for the activities, mainly the fresh water use. However, the addition of RAS must be prepared carefully both in terms of design and economy. The recirculation systems are generally fairly expensive to build and require training of staff for their operation (Losordo *et al.* 1998, Masser *et al.* 1999). Nevertheless, it may be an important alternative to improve the fish culture techniques used in hatcheries for brood stock and to develop good quality future fingerling production in Cuba.

The main objectives of this study were to compare water quality in a RAS with water quality in a limited reuse system (LRS) for Arctic charr culture; mainly focusing on the changes in concentration levels of some parameters of indicators of water quality as dissolved oxygen (DO), pH, carbon dioxide (CO₂), oxygen consumption (MO₂), total ammonia nitrogen (TAN), unionised ammonia (NH₃-N), nitrite nitrogen (NO₂-N), nitrate nitrogen (NO₃-N) and total suspended solids (TSS) of the inlet and outlet water at different points of each system to evaluate the performance of the RAS, taking into account:

- The oxygen demands of the fish.
- The production of metabolites by the fish.
- The removal of CO_2 by the aerators.
- The removal of ammonia by the biofilter.
- The removal of CO₂, TAN, NO₂-N, NO₃-N and TSS in wastewater (recirculating water).

2 LITERATURE REVIEW

Research and development in recirculating systems has been going on for nearly three decades. There are many alternative technologies for each process and operation. The selection of a particular technology depends upon the species being reared, site, infrastructure, production management expertise, and other factors (Dunning *et al.* 1998).

Noble and Summerfelt (1996) note that in aquaculture systems that reuse water, water quality should be maintained at levels sufficient for supporting healthy and fast growing fish. Operating a fish farm under limited water quality conditions can reduce the profitability of fish production, because the water quality problems can be lethal, lead to stress, and the resulting deterioration of fish health will reduce growth and increase the risk of infectious disease outbreaks and catastrophic loss of fish. The most common problems of water quality in RAS can be created by high or low water temperature, low DO levels, elevated waste metabolite concentrations, gas supersaturation, measurable dissolved ozone levels, and the presence of certain cleaning chemicals or chemotherapeutants in water (Twarowska *et al.* 1997).

2.1 Water quality in recirculation aquaculture systems (RAS)

2.1.1 Dissolved oxygen (DO) and carbon dioxide (CO_2) levels

Fish use oxygen to convert feed to energy and biomass. Depending upon species, according to Pillay and Kutty (2005), for optimum growth fish require a minimum DO concentration of approximately 5.0 mg L⁻¹ (warm water species) to 7.0 mg L⁻¹ (coldwater species). For salmonid species, the optimal levels of DO should be at least between 70-80% of oxygen saturation (not below 6.0 mg L⁻¹ and above 9.0 mg L⁻¹), oxygen saturation below this range decreases the maximal growth rate and higher saturation levels that exceed 120-140% can compromise the welfare of the fish causing oxidative stress and increased susceptibility to diseases and mortality (Aquafarmer 2004).

CO₂ is considered a toxic compound for fishes and is a limiting factor in intensive aquaculture systems where oxygen is injected into the inlet water while the water exchange rate is reduced; an increased CO₂ concentration in the culture water will reduce the CO₂ diffusion gradient between the fish blood and inspired water, and thus result in blood acidification, leading to a reduced arterial blood oxygen carrying capacity and a reduction in oxygen uptake (Sanni and Forsberg 1996).

In general, fish ventilate CO_2 (a by-product of metabolism) through their gills as molecular CO_2 gas, when the gas reacts with water they produce carbonic acid (H_2CO_3) , bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) and the equilibrium of the reactions depends on water pH values, in an inverse exponential relationship between CO_2 partial pressure and water pH values.

$$CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{-2}$$

The interdependence of pH, carbon dioxide, bicarbonate, and carbonate is illustrated in Figure 1 (Boyd 2000). The graph shows that below about pH 5, carbon dioxide is the only significant species of inorganic carbon, above pH 5, the proportion of bicarbonate increases relative to carbon dioxide until bicarbonate becomes the only significant species at about pH 8.3. Above pH 8.3, carbonate appears and it increases in importance relative to bicarbonate if pH continues to rise.

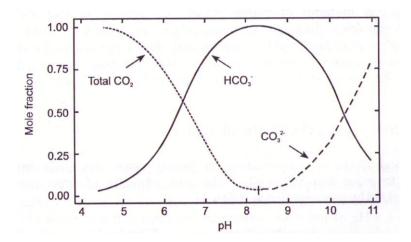


Figure 1: Effects of pH on the relative proportions of total CO₂, HCO₃, and CO₃². The mole fraction of a component is its decimal fraction of all the moles present (Boyd 2000).

Some studies of CO_2 excretion rates in salmonids have been conducted (Forsberg 1997), reporting CO_2 excretion rates of 2.8-3.0 mg CO_2 kg⁻¹ min⁻¹ from steelhead trout (*Oncorhynchus mykiss*) and coho salmon (*O. kitsutch*) and 1-2 mg CO_2 kg⁻¹ min⁻¹ from rainbow trout depending on the CO_2 levels present in the culture water.

The minimum DO concentration that is safe for fish is dependent on the concentration of dissolved CO₂ present in the water, the accumulated concentration of dissolved CO₂ within the culture tank will not be limiting (with no aeration or pH control) when the cumulative DO consumption is less than 10-22 mg L⁻¹, depending upon pH, alkalinity, temperature, and the species and life stage (Summerfelt *et al.* 2000).

The minimum safe DO level should be increased by 3-4 mg L⁻¹ if CO₂ concentrations are high, e.g. if dissolved CO₂ exceeds 30 mg L⁻¹ for salmonids or exceeds 40-50 mg L⁻¹ for certain warm water species. For example, dissolved CO₂ begins to effect salmonids at concentrations higher than 15-20 mg L⁻¹ in freshwater and less than 7-10 mg L⁻¹ in seawater, but many warm water species will tolerate considerably higher dissolved CO₂ levels in their environment such as cyprinids and hybrid striped bass.

Even the 20 mg L⁻¹ recommended as a safe level for salmonid culture may be conservative if DO concentrations in the water are at or above saturation levels (Summerfelt *et al.* 2000, Summerfelt *et al.* 2004), although as a precautionary approach, some authors such as Fivelstad *et al.* (1998) suggest that a maximum limit of CO₂ may be as low as 10 mg L⁻¹. For these reasons, DO is usually the first water quality parameter to limit culture tank carrying capacity.

2.1.2 Oxygen consumption (MO_2)

The oxygen consumption (MO_2) of fish is variable and depends on many factors such as temperature: MO_2 increases when temperature increases. Body mass: MO_2 has an inversely exponential proportion when the body mass increases. Feeding rate: MO_2 increases when the feeding rate increases due to the digestion of food. Growth rate has a directly proportional relationship with MO_2 . Swimming velocity and stress levels: increased stress levels may enhance the MO_2 of fish. The above factors are the most important that should be taken into account in any aquaculture system (Forsberg 1997, Timmons *et al.* 2002, Pillay and Kutty 2005).

The MO_2 of fish culture in tanks is calculated by the Fick equation, based on the DO concentration of the inflow and outflow water, the flow rate and the total biomass inside the tank. It is also possible to estimate oxygen requirements of fish based on feed intake.

Some authors have designed models to estimate MO₂ in salmonid species based on some factors such as body mass, temperature, water current velocity, time from feeding, water CO₂ levels and photoperiod (Fivelstad and Smith 1991, Forsberg 1994, Summerfelt *et al.* 2000). For example, Timmons *et al.* (2002) suggest, as a general rule for fish, that the ratio between MO₂ and feed intake, in units of mass, is around 0.25:1; this value is lower than values reported from studies of salmonids, where the MO₂ rate in this species fed to a maximum level is around 0.46-0.50:1 (Forsberg 1997). Timmons *et al.* (2002) also suggest, in general as respiratory quotient (the ratio of CO₂ produce when oxygen is consumed), that when 1.0 mg of oxygen per litre per minute is consumed by the fish, the fish can produce 1.3 mg of CO₂, and these values should be used for estimating expected CO₂ production in aquaculture systems; but in the case of salmonids, per 1.0 mg of DO consumed per litre they can produce 1.0 mg of CO₂ per litre (Aquafarmer 2004).

2.1.3 Nitrogen metabolites levels

2.1.3.1 Ammonia levels

The fish create and expel various nitrogenous waste products through gill diffusion, gill cation exchange, and urine and faeces excretion; in addition some nitrogenous wastes are accumulated from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere (Timmons *et al.* 2002). Ammonia exists in two forms: unionised ammonia (NH₃-N), and ionised ammonia (NH₄⁺-N), the sum of these two is called total ammonia nitrogen (TAN). The relative concentration of ammonia is primarily a function of water pH, salinity and temperature (Pillay and Kutty 2005).

The excretion of TAN by the fish varies depending on the species in culture. As a general rule, when 1.0 mg of oxygen per litre per minute is consumed by the fish, the fish can produce 0.14 mg of TAN (Timmons *et al.* 2002) and specifically for salmonids species, per 1.0 mg of DO consumed per litre they can produce 0.04-0.06 mg of TAN per litre (Aquafarmer 2004).

NH₃-N is the most toxic form of ammonia, so the toxicity of TAN is dependent on the percentage of the NH₃-N form in the TAN concentration. The proportion of NH₃-N increases if the pH increases and temperature or salinity decreases (Timmons *et al.* 2002), e.g. Fivelstad *et al.* (1995) found, in a short-term experiment, that intermediate salinities reduce the ammonia toxicity to Atlantic salmon smolts. Ammonia concentration levels are not a problem in a simple flow-through system but it is a problem when using recycling and reuse systems with biofilters to remove ammonia within the system. However, the fish farmers have to take care of the biofilters' functionality to maintain the acceptable ammonia concentration levels in the culture water depending of the culture species requirements (Aquafarmer 2004).

Unfortunately, NH₃-N can kill fish when it is above certain levels depending on the species (Table 1). For salmonids, long term exposure to concentrations between 0.05 to 0.2 mg L⁻¹ of NH₃-N can significantly reduce growth rate, fecundity and disease resistance and increase gill ventilation, metabolic rate, erratic and quick movements and can also cause mortality; due to the optimal conditions required for NH₃-N concentration levels in water has been less than 0.012 to 0.03 mg L⁻¹ for salmonids aquaculture (Summerfelt *et al.* 2004).

Table 1: Lethal levels of NH₃-N (concentration of nitrogen bound as NH₃) for some aquaculture species.

Specie	NH ₃ -N (mg L ⁻¹)	Reference
Rainbow trout	0.32	Timmons et al. 2002
Arctic charr	0.03	Aquafarmer 2004
Common carp	2.2	Summerfelt et al. 2004
Catfish	3.10	Summerfelt et al. 2004

Normally, warm water fish are more tolerant to ammonia toxicity than coldwater fish, and freshwater fish are more tolerant than saltwater fish, so in general, NH₃-N concentrations should be held below 0.05 mg L⁻¹ and TAN concentrations below 1.0 mg L⁻¹ for long-term exposure (Timmons *et al.* 2002). For Arctic charr culture, according to Aquafarmer (2004), the NH₃-N concentrations should be less than 0.025 mg L⁻¹ and TAN concentrations below 3.0 mg L⁻¹, keeping the pH levels below 8.0.

According to Forsberg (1997), the excretion of nitrogen is partitioned into two components: endogenous and post-pandrial or exogenous excretion rates. The endogenous nitrogen excretion (ENE) reflects catabolism and the turnover of body proteins, irrespective of the nutritional status of the fish. Post-pandrial excretion reflects the catabolism of proteins that originated from feeds. ENE usually ranges between 30-50 µg TAN kg⁻¹ min⁻¹ and 15-35 µg urea-N kg⁻¹ min⁻¹ for young salmonids species (Fivelstad *et al.* 1990, Forsberg 1997), these values indicate that around 80-90% of the nitrogen (TAN + urea-N) is excreted as ammonia. In the case of the post-pandrial excretion, Fivelstad *et al.* (1990), reported between 80-180 mg TAN kg⁻¹ days as average daily ammonia excretion rates from post-smolt Atlantic salmon fed maximum rates, which was equivalent to 22-33% of total nitrogen supplied. They also demonstrated with this study, that post-pandrial nitrogen excretion was linearly proportional to the nitrogen intake, even in fish fed limited rations. This general

pattern in salmonid species has also been demonstrated by other authors such as Beamish and Thomas (1984) and Forsberg (1997).

2.1.3.2 Nitrite (NO₂-N) and nitrate (NO₃-N) levels

Biofilters consist of actively growing bacteria attached to some surface(s), it can fail if the bacteria die or are inhibited by natural aging, toxicity from chemicals (e.g. disease treatment), lack of oxygen, low pH, or other factors. The biofilters take around 2 or 4 weeks to start functioning property after the bacteria population is established (Figure 2).

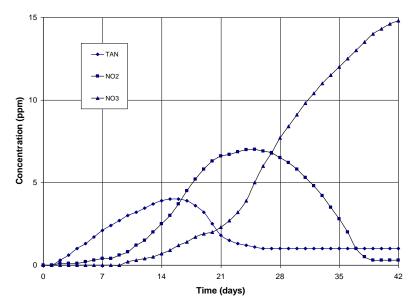


Figure 2: Typical startup curve for a biological filter showing time delays in establishing bacteria in biofilters (Timmons *et al.* 2002).

Nitrite and nitrate are produced when ammonia is oxidised by nitrifying bacteria concentrated within a biological filter, but they are also found throughout water columns and on surfaces within the recirculating system (Hagopian and Riley 1998). Non-biodegradable dissolved organic matter can also accumulate in the recirculating system water if it is degraded too slowly by the heterotrophic microorganisms in the biological filter.

According to Summerfelt and Sharrer (2004) biofilters contain both nitrifying bacteria and heterotrophic microorganisms that metabolise TAN and organic matter passing through the biofilter or trapped within the biofilter. The net results of the biofilter microbial respiration are a decrease in TAN, biodegradable organics, dissolved oxygen, alkalinity, and pH, and an increase in oxidation products of organics, as well as, NO₂-N, NO₃-N, and CO₂. Taking into account the overall stoichiometric relationship between subtracts and products produced during nitrification and nitrifier synthesis, nitrifying bacteria consume 4.6 mg L⁻¹ of oxygen while producing approximately 5.9 mg L⁻¹ of CO₂ for every 1.0 mg L⁻¹ of TAN consumed and 1.38 mg L⁻¹ of CO₂ are produced for every 1.0 mg L⁻¹ of dissolved oxygen consumed, when the respiration activity of nitrifying bacteria and heterotrophic microorganisms are considered together.

Nitrite is the intermediate product in the process of nitrification of ammonia to nitrate and it is toxic for the fish because it affects the blood haemoglobin's ability to carry oxygen oxidised the iron in the haemoglobin molecule from the ferrous state to ferric state. The resulting product is called methemoblobin, which has a characteristic brown colour, hence the common name "brown colour disease" (Timmons *et al.* 2002). The amount of nitrite entering the blood depends of the ratio of nitrite to chloride (Cl) in the water, in that increased levels of Cl reduce the amount of nitrite absorption. At least a 20:1 ratio of Cl: NO₂-N is recommended for channel catfish in ponds, tilapia and rainbow trout (Timmons *et al.* 2002, Pillay and Kutty 2005), levels below than 1.0 mg NO₂-N L⁻¹ are recommended for aquaculture systems (Pillay and Kutty 2005).

Nitrate (NO₃-N) is the end product of the nitrification process. As Timmons *et al.* (2002) note, NO₃-N is considered as the minimum toxic nitrogen product, with 96-h lethal concentration values more than 1000 mg NO₃-N L⁻¹ for some aquaculture species. In recirculating systems, NO₃-N levels are controlled by daily water exchanges, but in some systems with low water flow rates this parameter has become increasingly important and concentration levels should be lower than 10 mg NO₃-N L⁻¹ (Pillay and Kutty 2005).

2.1.4 pH levels, the relationship with nitrogen and inorganic carbon metabolites production in recirculation systems

The pH values express the intensity of the acid or basic characteristics of water. The pH scale ranges from 0 to 14, pH of 7.0 corresponding to the neutral point, while values of pH below 7.0 are acidic (the H⁺ ion predominates) and above 7.0, values are basic or alkaline (the OH⁻ ion predominates). The pH of most ground waters and surface waters are buffered by the inorganic carbon equilibrium system and they have pH values between 5.0 and 9.0 (Timmons *et al.* 2002).

Exposure to extreme pH values can be stressful or lethal for aquatic species, but it is the indirect effects resulting from the interactions of pH with other variables that depend on the water acid-base equilibrium such as dissolved CO₂, the relationship between NH₃-N and NH₄⁺-N levels and NO₂-N levels, that an increase of their concentrations depresses the pH values in water (Pillay and Kutty 2005). Low pH values increase the water solubility of some heave metals such as aluminium, copper, cadmium and zinc, their high concentrations in water cause toxic effects on fish, and also increase the toxicity of hydrogen sulphide on fish (Fivelstad *et al.* 2003). The higher toxicity levels of NH₃-N and CO₂ in water depends on the water's pH controls acid-base equilibrium; as an example, at 20°C and a pH of 7.0, the mole fraction of NH₃-N is 0.004, but at a pH of 10, the NH₃-N increase to 0.8 at the same temperature (Timmons *et al.* 2002).

In general, according to Aquafarmer (2004), the changes in pH water values should be less than 0.5 and pH values should be keept in a range of 6-9 for Arctic charr culture, depending to the water salinity and temperature used.

2.1.5 Solids concentration levels

Uneaten feed, feed fines, fish faecal matter, algae, and sloughed micro-biological cell mass are all sources of solids production within recirculating systems (Chen *et al.* 1993). Solids control is one of the most critical processes that must be managed in recirculating systems, because solids decomposition can degrade water quality and thus directly and indirectly affect fish health and the performance of other unit processes within recirculating systems (Chen *et al.* 1993). Suspended solids can harbour opportunistic pathogens and speed up the growth of bacteria. They are associated with environmentally-induced disease problems, and have been reported to cause sublethal effects such as fin rot and direct gill damage (Noble and Summerfelt 1996). Suspended and settleable solids may also affect reproductive behaviour, gonad development, and the survival of the egg, embryo and larval stages of fishes (Pillay and Kutty 2005).

For example, if solids are filtered and stored in a pressurised-bead filter (a type of granular media filtration unit) between 24-hr backwash cycles, as much as 40% of the TSS generated in the recirculating system may decay (Chen *et al.* 1993). The suspended organic solids common to recirculating aquaculture systems can exert a strong oxygen demand as they degrade into smaller particulate matter and leach ammonia, phosphate, and dissolved organic matter (Cripps 1995). The fine particles and dissolved compounds produced are considerably harder to remove when broken apart and dissolved than when they were contained within the original faecal or feed pellet (Chen *et al.* 1993). This dissolution process increases the water's oxygen demand as it deteriorates the water quality within the recirculating system and in the discharged effluent.

Some authors such as Timmons *et al.* (2002) and Pillay and Kutty (2005) had considered TSS concentrations less than 80 mg L⁻¹, in general as water quality criteria for aquaculture, but in the case of sensitive species like salmonids, Aquafarmer (2004) suggests to maintain the TSS concentrations around 4.5 mg L⁻¹ to keep the values on the safe side and fix as a concentration limit 15 mg L⁻¹.

Therefore, water quality should be monitored closely in a recirculating system so those problems with the water treatment units can be detected early and corrected. Water quality is also of concern if the effluent characteristics (e.g. biochemical oxygen demand, suspended solids, phosphorus, or nitrogenous compounds) of the culture facility must be controlled to meet water pollution requirements (Timmons *et al.* 2002).

2.2 Arctic charr as a farming species in Iceland

Arctic charr is a salmonid specie that can live in different environments depending on its life stage (freshwater, brackish and marine water between 30 - 70 m of depth). The Anadromous forms spend a considerable time of their lives at sea; non-migratory populations remain in lakes and rivers. The freshwater populations feed on planktonic crustaceans, amphipods, mollusks, insects and fishes and they are extremely sensitive to water pollution (cold water and oxygen oriented) in natural and captivity conditions (Aquafarmer 2004).

Around 1930 the farming of trout grew in Denmark, with farming of rainbow trout ensuing, which is now widely practised. In 1970 the growing of North Atlantic salmon took off in Norway with massive production that increases every year, as the conditions for farming salmon in sea-cages in the Norwegian fjords are excellent. Other countries and regions extensively farming North Atlantic salmon are Chile, Scotland, Ireland, the Faroe Islands, Canada, USA and Tasmania (Pillay and Kutty 2005). The farming of Arctic charr has been practised for quite some years, but never on a large scale.

Why is it desirable to develop the Arctic charr culture in Iceland? As Aquafarmer (2004) notes, Arctic charr for farming is a good choice at colder climates for various reasons:

- The access to suitable cold and clean water resources used for the culture activities.
- Arctic charr does well in cool waters because it is an indigenous species in the northern hemisphere and grows much faster at low temperatures than other salmonid species kept for farming.
- It is possible to keep Arctic charr at a greater density than many other fish species, thus making more efficient use of the farming space. Actually Arctic charr seems to grow better at 50 kg m⁻³ than at 15 kg m⁻³.
- The Arctic charr is robust and easy to farm. It tolerates handling well and shows good resistance to many diseases. Losses are usually minor after the initial period of the embryonic stage.
- Its use of feed is good as the Arctic charr takes feed from the bottom of the tank and also eats in the dark night time.
- Arctic charr has marketable qualities such as delicate taste, attractive colour, low-fat meat and its market size is from one portion size up to two kilograms.

But there are also some disadvantages, such as:

- The charr is prone to become sexually mature already in the second year. At sexual maturity the growth rate markedly decreases and the quality deteriorates. Sexually mature fish therefore cannot be considered a marketable product.
- There is considerable variability in the growth rate depending on the season. Great size variance of fish in the same tank can create marketing problems.
- The colour of the flesh can be variable within a group. Usually the buyers want their fish strongly pink.

The commercial Arctic charr market is dominated by four producing countries: Iceland, with more than 900 tons per year is considered the major producer in Europe; Norway and Sweden, they are producing considerably less than Iceland; and Canada with less than 400 tons per year. Several other countries including Scotland, Ireland, France and Denmark are still minor producers. Including the production from the remaining countries, the total Arctic charr production is around 1800 – 1900 tons per year (Aquafarmer 2004). The main charr products for the market are either head-on frozen and gutted, or head-on chilled and gutted. At present, the price of charr is approximately ISK 380-500 for gutted fish and ISK 600-900 for fillets and in Canada prices are in the \$4.50–5.0/lb range (Aquafarmer 2004).

3 MATERIALS AND METHODS

In the present study an experiment was conducted in Verid, the Aquaculture Research Facilities of Holar University College, Iceland, during 4 weeks. Two different systems were compared in the experiment: a RAS with a biofilter and a LRS. The net water used in the LRS was 0.2 L min⁻¹ kg⁻¹ which is similar to the water used in Icelandic charr farms. The net water used in the RAS was initially the same as the LRS (0.2 L min⁻¹ kg⁻¹) and then it was gradually adjusted to 0.008 L min⁻¹ kg⁻¹ so that the water quality was within acceptable levels. Each system had two culture tanks (800 L), a reservoir tank, water pump, sand filter and aerator. The RAS includes a biofilter unit while the LRS does not have a biofilter (Figure 3). Arctic charr with an average body mass of around 190 g ind.⁻¹ were used. The initial stocking density was 157 individuals in each tank (40 kg m⁻³), and 20 ppt of water salinity at 10°C of temperature and DO levels were kept between 100-115% of saturation (≈ 9.84-11.05 mg L⁻¹).

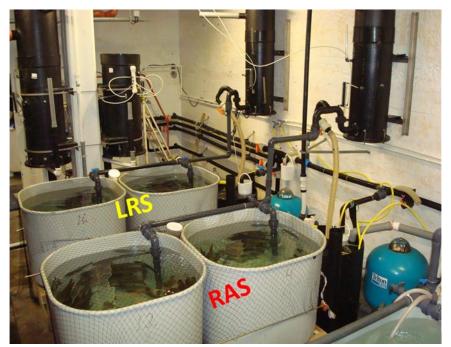




Figure 3: Aquaculture systems used for the experiment. Limited reuse system (LRS) and recirculating aquaculture system (RAS) with biofilter.

The water temperature, DO, salinity and pH were measured daily in each system in each of measurement point as show in Figure 4. The water temperature and DO water levels were measured with YIS-550A DO meter, the water salinity was measured with a PAL-06S refractometer (Atago Company) and the pH by OxyGuard pH meter. The total fish biomass of each tank in each system was measured per 2 weeks.

Water samples were collected to measure the concentrations of CO₂, TAN and TSS (3 replicas per measuring per parameter) in each system two times per week at the

measurement point as show in Figure 4, and the NO₂-N and NO₃-N concentration levels were also measured in the water samples taken from the biofilter outlet water (point 5) in the RAS two times per week.

The water samples were analysed in the laboratory of Verid to determinate CO₂, TAN and TSS concentrations according to the Standard methods for evaluation of water and wastewaters referred by Danish Standard Methods DS 224 (1975), APHA (1998) and Timmons *et al.* (2002). These methods are:

CO₂: CO₂ was measured with the single acid addition method. First, the initial temperature and salinity of the samples was measured. Then the samples were stored at 25°C for at least 1 hour for the samples to reach this temperature. Finally, 100 mL of sample was measured accurately with a pipette and placed in a beaker, the temperature and pH of the sample was recorded. Then 25 ml (for samples with full salinity but only 5 to 10 ml for fresh water samples) of standanised 0.01 M HCl was added to the sample while mixing thoroughly. The resulting pH was recorded. The total inorganic carbon (TIC) and CO₂ concentrations were calculated using the programme CO₂ sys.exe program with the NBS scale option. It was assumed that the carbonic alkalinity reflected the total Alkalinity (TA) of the sample.

TSS: A well – mixed sample (? Volume) was filtered through a weighed standard glass fibre filter (Whatman GF/C). Then the filter was dried at 105°C for at least one hour and the dry weight of the filter measured. The difference in the weight increase of the filter divided by the total sample volume filtered represents the total suspended solids concentration in the sample.

TAN: TAN was measured colorimetrically by indophenol blue method as describe in the Danish Standard methods DS 224 (1975). A 25 ml sample was measured into a reaction flask. Then 1.0 ml of sodium citrate solution, 1.2 mol L⁻¹, 1.0 ml of reagent A and 1.0 ml of reagent B were added in succession. The reagents should be prepared before the start of the measurements as shown in the technique DS 224. The samples were mixed well. The reaction flask was closed and left for two hours for the colour to develop in a dark place. The absorbance of the sample was measured at 630 nm in a spectrophotometer at latest 24 hours after mixing using 10 mm cuvettes. The TAN concentration was calculated using the calibration curve equation previously established.

The NO₂-N and NO₃-N concentration levels were measured using reagent test kits for Nitrite (CHEMets[®] Kit Nitrite K-7004) and Nitrate (CHEMets[®] Kit Nitrate K-6904) acquired from CHEMetrics Company, USA.

The oxygen consumption was calculated from each measurement in each system as:

$$MO_2 = (DO_{in} - DO_{out}) * Q / Bt$$
 (1)

where MO_2 is the oxygen consumption rate (mgO₂ min⁻¹ kg⁻¹), DO_{in} and DO_{out} are the dissolved oxygen concentrations (mg L⁻¹) in the inlet and outlet water, Q is the water flow inside the tanks (L min⁻¹) and Bt is the total fish biomass per tank (kg).

The rate of removal and addition of CO₂, TAN, NH₃ and TSS, were calculated as:

$$SX = (X_{out} - X_{in}) * Q / Bt$$
 (2)

where SX is the rate of either CO_2 , TAN, NH_3 and TSS (mg min⁻¹ kg⁻¹), X_{out} and X_{in} are the outlet and inlet concentration (mg L⁻¹) of each metabolite, Q is the water flow inside the tanks (L min⁻¹) and Bt is the total fish biomass per tank (kg).

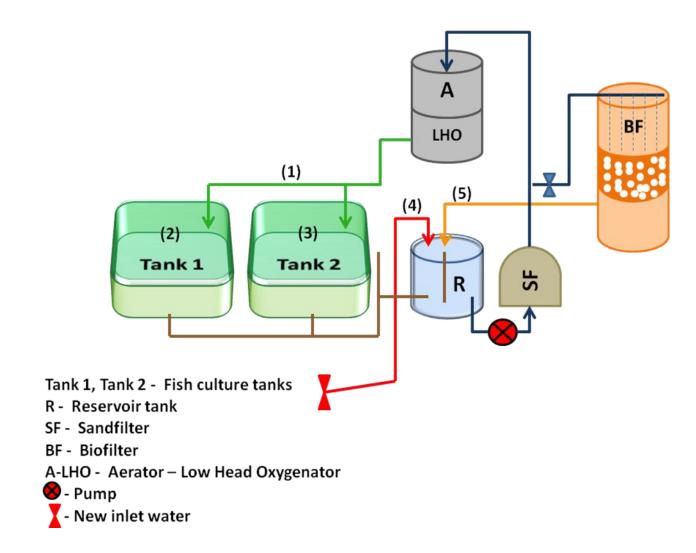


Figure 4: General diagram of the systems and measurement points. Recirculating aquaculture system (RAS) with biological filter coupling and limited reuse system (LRS) without biological filter, where (1) inlet water after total treatment, (2) fish culture tank 1, (3) fish culture tank 2, (4) inlet new water and (5) outlet water from BF.

4 RESULTS

4.1 Dissolved oxygen (DO) levels and oxygen consumption (MO₂) in the systems

The variation rates in DO concentrations and the rate of MO_2 in both systems during the experimental time are shown in Figure 5. The DO concentrations in the outlet water from the tanks in the LRS varied between 7.45-10.0 mg L⁻¹, while the inlet water tanks ranged between 8.90 and 11.89 mg L⁻¹. For the RAS, the DO concentrations ranged between 8.09 and 9.78 mg L⁻¹ for the outlet water and 9.77-11.15 mg L⁻¹ for the inlet water. The DO concentration was similar in both systems and higher than the recommended levels for salmonid aquaculture. The oxygen consumption (MO_2) in both systems was similar (Figure 5). The mean oxygen consumption in the LRS was 2.07 mg O_2 min⁻¹ kg⁻¹ ranging between 0.73 and 3.07 mg O_2 min⁻¹ kg⁻¹ and in the RAS the mean oxygen consumption was 1.80 mg O_2 min⁻¹ kg⁻¹ ranging between 0.58 and 2.62 mg O_2 min⁻¹ kg⁻¹. The total body mass was 59.27 kg and 58.45 kg in the LRS and RAS respectively.

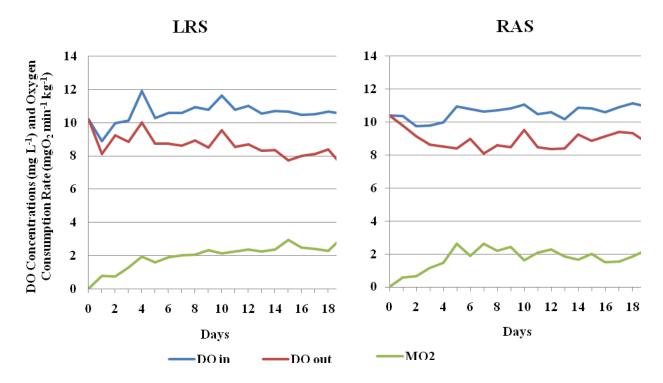


Figure 5: Dissolved oxygen (DO) concentrations (mg L^{-1}) in the water inlet tanks and in the outlet water from the tanks and the oxygen consumption rate (MO₂) of the fishes (mg O₂ min⁻¹ kg⁻¹) in each system during the experimental time.

4.2 pH water levels in the systems

In both systems, the pH of the new water entering the systems and the inlet water into the tanks was similar, ranging from 7.4-7.8 and 7.7-8.0 for the LRS and RAS respectively (Figure 6). The pH for day 0 (7.98 for the LRS and 8.01 for the RAS) show values without fish in the systems. The pH in the outlet from the tanks was

lower than the pH of the inlet water ranging from 7.41-7.64 (mean 7.55) for the LRS and 7.43-7.80 (mean 7.58) for the RAS.

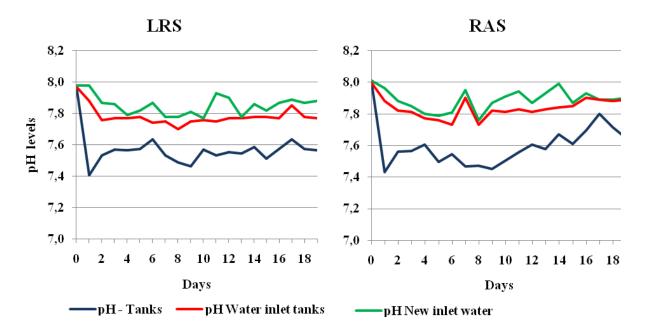


Figure 6: pH levels in the tanks water, in the water inlet tanks and in the new inlet water to the system for each system during the experimental time.

4.3 Total inorganic carbon (TIC) and carbon dioxide (CO_2) levels in the systems: removal rate of carbon dioxide (CO_2)

The concentration of TIC was similar in the inlet water to the systems and in the outlet from the tanks (Figure 7) and appears to be primarily determined by the TIC concentration in the inlet water. The TIC concentrations in all measuring points were $51.10-90.12 \text{ mg L}^{-1}$ in the LRS and $66.70-91.89 \text{ mg L}^{-1}$ in the RAS.

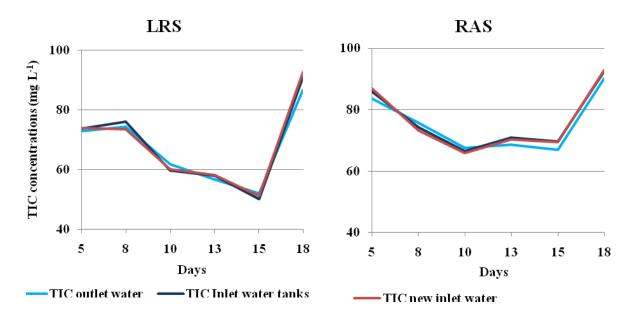


Figure 7: Total inorganic carbon (TIC) concentrations (mg L⁻¹) in the outlet and inlet water tanks and in the new inlet water to the system for each system during the experimental time.

The CO_2 concentrations were similar in the LRS and in the RAS (Figure 8). The mean CO_2 concentration in the inlets into the tanks was 2.01 mg L⁻¹ in the LRS and 1.87 mg L-1 in the RAS. The CO_2 concentration in the outlet from the tanks was 1.87-4.32 mg L⁻¹ in both systems and the mean values were 3.21 and 3.10 mg L⁻¹ for the LRS and RAS respectively (Figure 8). During the last stage of the experiment the CO_2 concentration in the outlet from the tanks was lower in the RAS than in the LRS.

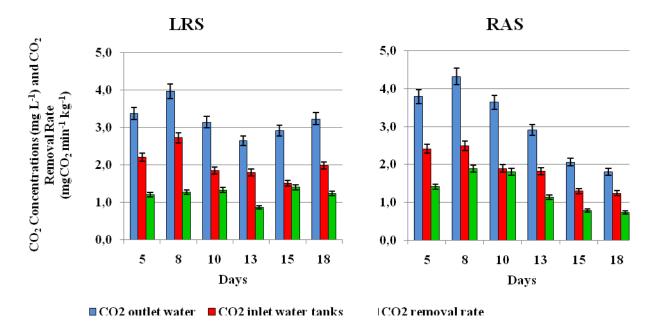


Figure 8: Carbon dioxide (CO₂) concentrations (mg L⁻¹) in the outlet water from the tanks and in the inlet water tanks and CO₂ removal rate from the system (mgCO₂ min⁻¹ kg⁻¹) for each system during the experimental time.

4.4 Nitrogen metabolites

4.4.1 Total ammonia nitrogen (TAN) concentrations and removal rate of TAN in the systems

The TAN concentrations were higher in the RAS than in the LRS system (Figure 9). In both systems the TAN concentration increased over time albeit more in the RAS system. The TAN concentrations in the LRS were 0.163-0.482 mg L^{-1} in the outlet water from the tanks 0.149-0.447 mg L^{-1} for the water inlet to the tanks. In the RAS the TAN concentration in the outlet from the tanks was 0.251-1.520 mg L^{-1} and 0.246-1.577 mg L^{-1} .

The estimated TAN removal rate in the RAS (calculated from TAN concentration in the inlet water and outlet water to the tanks) was 0.5 to -5.7 mg TAN min⁻¹ kg⁻¹. In the RAS, the TAN concentration was consistently higher in the inlet into the tanks than in the outlet resulting in negative estimates of removal rate (Figure 8). This may suggest

that TAN is also produced in other parts of the system. In fact, it was later discovered that the sand filter was not flushed adequately and that some TAN appeared to emanate from the filter. The TAN removal rate in the LRS was $0.7-1.5 \text{ mg TAN min}^{-1} \text{ kg}^{-1}$.

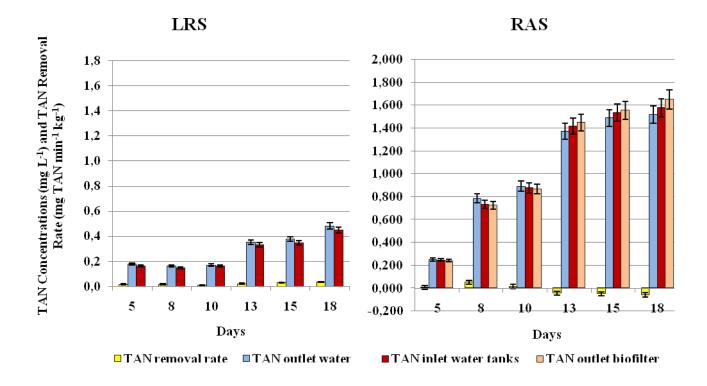


Figure 9: Total ammonia nitrogen (TAN) concentrations (mg L⁻¹) in the outlet water from the tanks and in the inlet water tanks and TAN removal rate (mg TAN min⁻¹ kg⁻¹) for each system during the experimental time.

To examine the reason for the high TAN values in the inlet into the tanks, samples were taken on day 26 from the inlet into the biofilter in addition to samples from the inlet into the tanks and from the outlet (Figure 10). The outlet water from the tanks goes through a hydrocyclone and then to a reservoir and then it is pumped through a sand filter (Figure 4). From the sand filter the water goes either to the aerator or to the biofilter and then back to the reservoir. From day 0 samples were taken from the inlet to the tanks, from the outlet and from the inlet of new water to the system. On day 26, further samples were taken from the inlet into the biofilter. The TAN concentration in the water entering the biofilter was higher than in the inlet water and in the outlet of the tanks (Figure 10). This suggests that TAN is added to the water in the hydrocyclone, the reservoir or in the sand filter. After the sand filter was flushed, the TAN concentration at the inlet of the biofilter was reduced (Figure 10) suggesting that the high TAN concentration did in fact originate from the sand filter.

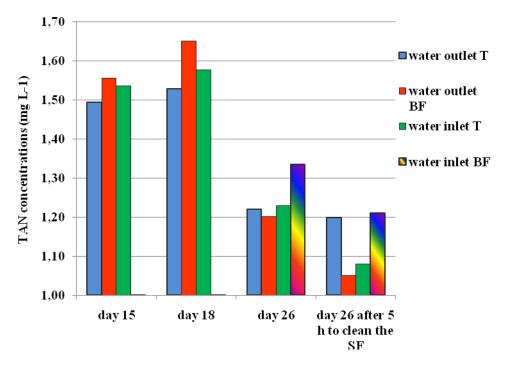


Figure 10: TAN concentration levels in different water points in the RAS at days 15 and 18 of the experimental period and at day 26, one week after the end of the experiment, before and after 5 hours to clean the sand filter.

4.4.2 Unionised ammonia (NH₃-N)

In general, the NH_3 -N concentration in the systems reflected the TAN concentration, increasing during the experimental period in both systems (Figure 11). The NH_3 -N concentrations were lower in the LRS than in the RAS (Figure 11). The NH_3 -N concentrations in the RAS were close to 0.025 mg L^{-1} , which is the maximum recommended level for salmonid aquaculture. In the LRS, the NH_3 -N concentrations were 0.001-0.003 mg L^{-1} and 0.001-0.005 mg L^{-1} in the water inlet to the tanks during all the experimental period. The NH_3 -N concentrations in the RAS were 0.001-0.014 mg L^{-1} in the outlet from the tanks and 0.002-0.018 mg L^{-1} in the outlet water from the biofilter unit and 0.003-0.023 mg L^{-1} in the water inlet to the tanks.

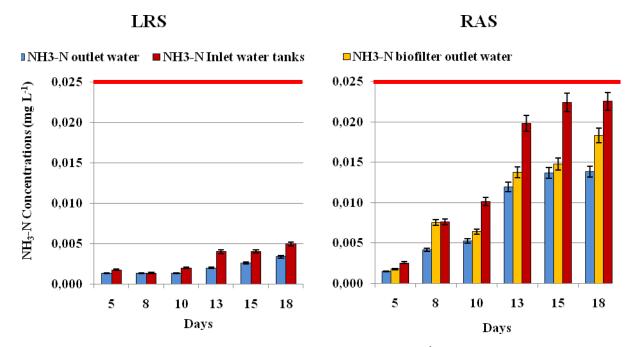


Figure 11: Unionised ammonia (NH₃-N) concentrations (mg L⁻¹) for each system in the outlet water from the tanks and in the water inlet tanks and in the outlet water from the biofilter in the RAS, during the experimental time. The red line in both charts indicates the unionised ammonia (NH₃-N) concentrations limit of water quality (mg L⁻¹) for salmonids culture.

4.4.3 Nitrogen metabolites

The nitrite concentration in the RAS increased during the experiment with a concomitant increase in nitrate concentration (Figure 12). The TAN concentration was higher than either the nitrite or nitrate concentration during the experiment. The NO₂-N and NO₃-N concentrations started to increase on day 8 and 10 ranged from 0-1.10 mg L⁻¹ and 0-0.66 mg L⁻¹ respectively. This pattern of increase in TAN, NO₂-N and NO₃-N suggests that the function of the biofilter was gradually increasing during the experiment.

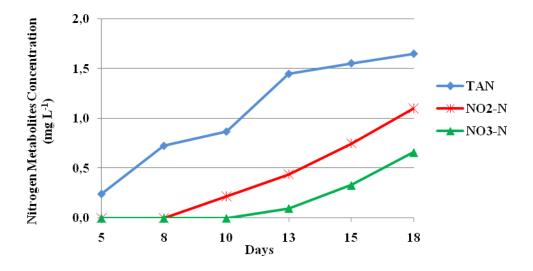


Figure 12: Nitrogen metabolites (TAN, NO₂-N and NO₃-N) concentrations (mg L⁻¹) in the outlet water from the biofilter in the RAS.

The function of the biofilter was tested by turning it off for one hour while the concentration of TAN was measured (Figure 18). Samples were taken from the water outlet from the tanks and from the water inlet to the tanks. The TAN concentrations increased after the biofilter was turned off by 0.1 mg L⁻¹ and 0.2 mg L⁻¹ in the outlet and inlet water respectively (Figure 18).

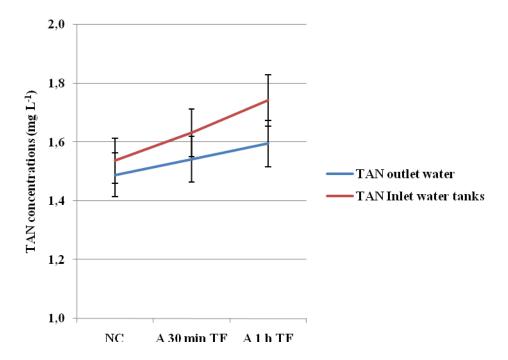


Figure 13: Total ammonia nitrogen (TAN) concentrations (mg L⁻¹) in the outlet water from the tanks and in the inlet water tanks for the RAS during three stages at the same experimental day (18), where NC (normal conditions), A 30 min TF (after 30 minutes of turn off the biofilter) and A 1 h TF (after 1 hour of turn off the biofilter).

4.5 Total suspended solids (TSS) levels and removal rate of TSS in the systems

The TSS concentrations in the outlet and inlet water performance increased during the experiment (Figure 14). The TSS concentration in the outlet water from the tanks in the LRS was $1.04-5.58 \text{ mg L}^{-1}$ and $0.93-8.85 \text{ mg L}^{-1}$ in the RAS. The TSS in the inlet water tanks was 0.10-4.47 and $0.70-8.75 \text{ mg L}^{-1}$ respectively. However the TSS was higher in the RAS than in the LRS.

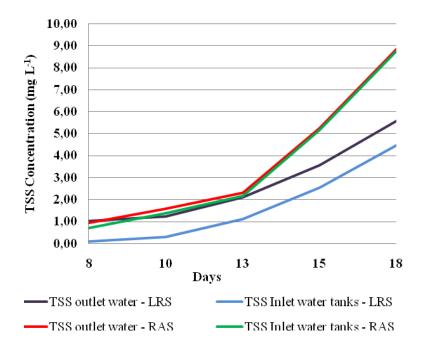


Figure 14: Total suspended solids (TSS) concentrations (mg L⁻¹) in the outlet water from the tanks and in the inlet water tanks for each system (LRS and RAS) during the experimental time.

The TSS removal rate in the systems (Figure 15) was different for each one. The LRS showed higher values of TSS removal rate than the RAS during all the experimental period with values between 96-110% and a relatively constant performance, while the RAS values were between 23-10% of TSS removal rate, however lower values were obtained at the end of the experiment than at the beginning.

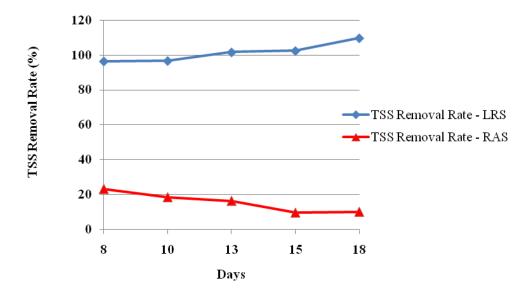


Figure 15: Total suspended solids (TSS) removal rate (%) for LRS and RAS during the experimental time.

5 DISCUSSION

5.1 Dissolved oxygen (DO) levels and oxygen consumption (MO_2) in the systems

In any aquaculture system the DO concentrations in the culture water is one of the most important parameters to maintain at safe levels to provide optimal conditions for the fish (Timmons *et al.* 2002, Pillay and Kutty 2005). The DO concentrations in the inlet water were over 8.9 mg L⁻¹ and over 7.45 mg L⁻¹ in the outlet water in both systems. These values are higher than the 7.0 mg L⁻¹ suggested for salmonid aquaculture (Pillay and Kutty 2005).

Aquafarmer (2004) also recommends, for salmonid culture, levels of DO saturation between 70-80% for normal conditions (0 ppt of salinity and 6°C of temperature) and the DO levels in both systems exceeded this value.

The fish oxygen consumption rate (MO_2) in both systems was similar, increasing during the first 4 days of the experiment while the fish adapted to new water conditions and thereafter they were fairly constant or 2.00-3.07 mg $O_2 \, \text{min}^{-1} \, \text{kg}^{-1}$ in the LRS and 2.00-2.62 mg $O_2 \, \text{min}^{-1} \, \text{kg}^{-1}$ in the RAS. The MO_2 was comparable to what has been reported for 200-300 g Arctic charr in other studies (Summerfelt and Sharrer 2004, Summerfelt *et al.* 2004a).

The MO₂ of the fish in the LRS was a little higher than in the RAS. The MO₂ of fish is variable and depends on many factors such as temperature, body mass, feeding rate, growth rate, stress and other factors (Forsberg 1997, Timmons *et al.* 2002, Pillay and Kutty 2005). The initial biomass in both systems was approximately 60 kg at the beginning of the experiment. During the experiment the biomass was reduced slightly because some fish died. The final biomass was slightly higher in the LRS than in the RAS.

The growth rate of the fish was also slightly higher in the LRS than in the RAS. The biomass increased by 0.110 kg day⁻¹ in the LRS and only 0.035 kg day⁻¹ in the RAS (see Appendix from daily measurements in the systems). Moreover, the feed intake of the fish in the RAS was lower than in the LRS.

The concentration of nitrogen metabolites was higher in the RAS and this may have contributed to higher stress levels in this system and that may have caused the fish to lose their appetite, reduce growth rates and the total body mass in the RAS system. This may also have contributed to the lower MO₂ of the fish in the RAS.

5.2 pH levels in the systems

The pH levels depend on the performance of the total inorganic carbon equilibrium in the water and which one of carbon species is predominant in the water environment as was shown in section 2, Figure 1 (Boyd 2000). The pH levels of the culture water were 7.4-8.0 in both systems during all the experimental period, values which are within the optimal rate for Arctic charr aquaculture (Aquafarmer 2004).

The pH value fluctuations observed in both systems during the period (Figure 6) depended on the total inorganic carbon (TIC) concentration of the new water in the inlet to the systems (Figure 7) and on the total CO₂ concentration in the culture water (Figure 8). The low pH levels registered in the outlet water from the culture tanks for the LRS during the whole period in comparison with the pH levels from the RAS were mainly due to the higher CO₂ concentration levels in the RAS (Fig. 8).

The pH values in the inlet water were higher than in the outlets in both systems (Figure 6) due to the function of the aerators. They removed the dissolved CO₂ from the water and, as a result, the water pH increased.

5.3 Total inorganic carbon (TIC) levels and carbon dioxide (CO_2) levels in the systems: removal rate of carbon dioxide (CO_2)

Carbon dioxide (CO₂) is a function primarily of the total amount of inorganic carbon (TIC) present in water and of pH (Summerfelt *et al.* 2000). During the experiment, the TIC concentrations measured for the outlet and inlet water from the tanks and for the new water inlet to the systems were similar and depended mainly on the TIC concentration in the new inlet water in the systems. The TIC was higher in the RAS than in the LRS during whole the period analysed. This may be related to the slightly higher temperature in the RAS system (see Appendix).

The CO₂ concentrations were similar in both systems during the entire experiment (Figure 8). The CO₂ concentration was slightly higher in the LRS (2.64-3.97 mg CO₂ L⁻¹) and than in the RAS (1.87-4.32 mg CO₂ L⁻¹). This difference may be a result of the lower metabolic rate of the fish in the RAS. The amount of CO₂ produced for each mg of oxygen consumed was about 1:1 as had been suggested by other studies in Arctic charr (Aquafarmer 2004, Forsberg 1997). The CO₂ concentrations in both systems were lower than the 10-20 mg L⁻¹ which is the suggested limit for CO₂ in salmonid aquaculture (Fivelstad *et al.* 1998, Summerfelt *et al.* 2000, Summerfelt *et al.* 2004).

The low dissolved CO_2 in both systems suggests that the aerators effectively removed CO_2 from both systems (Figure 8).

5.4 Total ammonia nitrogen (TAN) and unionised ammonia (NH₃) levels in the systems: removal rate of TAN

During the study, the TAN concentrations were higher in the RAS than in the LRS (Figure 9).

The TAN concentrations in the LRS are mainly determined by water exchange. The net water inflow into the LRS was 0.2 L min⁻¹ kg⁻¹ during the entire experiment, it indicates that the system changes the total water volume 10 times per day. The total TAN production in the system was approximately 6.0 mg min⁻¹ (12 L min⁻¹ x 0.5 mg L⁻¹). This suggests that the TAN production was about 0.05 mg kg⁻¹ min⁻¹ which is comparable with the expected Arctic charr TAN production for each mg of oxygen consumed (0.04-0.06:1). The high water exchange rate maintained the TAN

concentration lower than 3.0 mg L⁻¹ which is recommended for good water quality for Arctic charr culture (Aquafarmer 2004).

The initial water exchange in the RAS was similar to the LRS and, therefore, the initial TAN concentration was similar in both systems. Then the water exchange was reduced in the RAS up to 0.05 L min⁻¹ kg⁻¹ on day 6 of the experiment and then the TAN concentration increased. However, the TAN production in the RAS at this time was around 0.2 mg kg⁻¹ min⁻¹, approximately 4 times higher than the LRS. During this period the major percentage of TAN was removed from the system both by water exchange and through the biofilter.

Thereafter the water exchange reduced up to 0.008 L min⁻¹ kg⁻¹ in the RAS on day 12 of the experiment, the TAN production in the system increased up to 1.25 mg kg⁻¹ min⁻¹, around 25 times higher than the LRS and than the beginning of the experimental period for this system. During this last period, the TAN concentrations showed a difference in performance from the beginning, higher values were obtained for the water inlet to the tanks and from the water outlet biofilter than the water outlet from the tanks (Figure 9). However, the apparent TAN removal rate obtained during this time showed negative values. Its performance should be due to the influence of various factors in conjunction: the reduction of the exchange flow rate up to 40%, the possibility of ammonia production in some places between the tanks and the biofilter, and the biofilter capacity to remove ammonia.

The outlet water from the tanks goes to the reservoir tank and from there it is pumped to the sand filter. From the sand filter, part of the water goes to the biofilter and returns again to the reservoir while the remaining water goes to the aerator and then enters the tanks. During the experiment, the water coming from the sand filter was not sampled. Apparently, some TAN was produced in the sand filter thus increasing the TAN concentration in the system.

When the biofilter was turned off on day 18, the TAN concentration increased by about $0.2 \text{ mg L}^{-1} \text{ hour}^{-1}$. This suggests that the biofilter was removing approximately 0.003 mg of TAN L⁻¹ min⁻¹ (5.49 mg of TAN min⁻¹) or about 7.32% of the TAN produced in the system.

On day 26, after the end of the experimental time, the TAN concentration was measured in the water inlet to the biofilter and in the other measurement points in the RAS both before and after the sand filter was flushed. The results showed that the water inlet to the biofilter had higher TAN concentration values than the water outlet from the tanks before flushing the sand filter; and after it was flushed, the water inlet to the tank almost had the same TAN values as the water outlet from the tanks, and TAN concentrations in the water outlet biofilter were reduced considerably. This clearly demonstrates the need for a regular back flush of the sand filter to avoid build up of heterotrophic bacteria culture which produces ammonia that can compromise the performance of the biofilter and the suitable operation of the system.

The NH₃-N concentration levels were lower than 0.025 mg L⁻¹ during the whole period for both systems, in the optimal rate for NH₃-N levels recommended for Arctic charr culture (Aquafarmer 2004), although it is important to draw attention to the NH₃-N levels obtained for the RAS at the end of the experimental period, where the

NH₃-N concentrations in the inlet water tanks showed values in close proximity to 0.025 mg L⁻¹ (Figure 11).

5.5 Biofilter performance in the RAS

The concentrations of nitrite and nitrate were measured during the experiment. According to Timmons *et al.* (2002), the biofilter goes through several stages while the nitrifying bacteria are multiplying and reaching full capacity. First the TAN concentrations increase but when the activity of the nitrifying bacteria increases first the NO₂-N increases. Then the NO₃-N concentrations increase while the TAN and NO₂-N begin to decrease (Fig. 2).

As shown in Figure 12, the TAN concentration in the RAS was higher than the other nitrogen metabolite concentrations, suggesting that the biofilter was still maturing. Because of the short duration of the experiment (3 weeks) it was not possible to observe the full development of the biofilter; but with the biofilter TAN removal rate values obtained during the last experiments on days 18 and 26, it was demonstrated that the biofilter was removing ammonia out the system but not in high enough amounts to keep the TAN concentrations out of the water outlet from the biofilter and the water inlet tanks lower than the TAN concentrations of the outlet water from the tanks in the system, due to the ammonia contribution from the sand filter.

5.6 Total suspended solid (TSS) levels in the systems: removal rate of TSS

Waste solids control is one of the most critical processes that must be managed in recirculating systems, it accumulates in aquaculture systems from uneaten feed, feed fines, fish faecal matter, algae, and biofilm cell mass sloughed from biofilters (Timmons *et al.* 2002). Solids decomposition can degrade water quality and thus directly and indirectly affect fish health and the performance of other unit processes within recirculating systems such as elevated organic matter in the sand filters and inhibition of the bacteria process within biofilters (Chen *et al.* 1993) because they are a major source of carbonaceous oxygen demand and nutrient input into the water (Timmons *et al.* 2002).

In this study, the TSS concentrations in the outlet and inlet water for both systems showed the same performance (Figure 14), they increase with the time from the beginning until the end of the experiment, and the concentration was lower in the LRS than the RAS during the whole period (Figure 14) as a result of the TSS removal rate in the systems (Figure 15). However, the TSS concentrations in both systems were lower than the 15 mg L⁻¹ recommended for Arctic charr (Aquafarmer 2004).

Suspended solids within the fish culture tanks are very naturally difficult to remove because they do not settle out by conventional gravity settling basins and therefore a treatment process and/or high exchange flow rate is required (Timmons *et al.* 2002, Pillay and Kutty 2005). The TSS removal rate in the LRS was higher than the RAS during the entire experimental period, and for the RAS lower values were obtained at the end of the experiment. Differences between systems and within the RAS were caused by the water exchange flow rate for the system (Timmons *et al.* 2002). For the LRS the TSS removal rate values obtained showed a constant performance due to the

constant net flow rate in the system (0.2 L min⁻¹ kg⁻¹) with an exchange of the total water volume out of the system 10 times per day during the whole period studied, while the RAS, at the beginning the total water volume exchange rate was the same as the LRS and thereafter decreased gradually to 0.4 times per day when the net water flow was reduced to 0.008 L min⁻¹ kg⁻¹. Thus the changes in the net water flow for the RAS caused a gradual increase in the TSS concentrations within the system reducing the capacity of it to remove the TSS produced.

6 CONCLUSIONS

- The water quality parameters measured were well within the acceptable levels for Arctic charr culture.
- The water quality was better in the LRS than in the RAS during the experimental time.
- The biofilter unit in the RAS started to work around a week later than the normal performance referred to in the literature due to the lower temperatures used for the Arctic charr culture in the experiment.
- The sand filter should be cleaned regularly, 2 or 3 times per week, to avoid build up of heterotrophic bacteria culture which produces ammonia and can affect the performance of the RAS.

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APPENDIX: TABLES OF MEASUREMENTS.

Tables of Measurements for the Limited Reuse System (LRS) $\,$

Table 2: Daily measurements in the LRS tank No. 1 between days 0 - 9.

Days	0	1	2	3	4	5	6	7	8	9
Date	21.1.2008	22.1.2008	23.1.2008	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008
Temperature (°C)	9,9	9,5	9,9	9,9	9,9	10,5	10,3	10,2	10,1	10,6
pН	7,97	7,41	7,54	7,56	7,55	7,58	7,63	7,54	7,49	7,47
Salinity (ppt)	20	20	20	20	20	20	20	20	21	20
DO in (%)	104,2	88,6	99,3	102,4	120,0	101,8	106,6	106,2	109,7	108,8
DO in (mg L ⁻¹)	10,20	8,90	9,95	10,10	11,89	10,26	10,58	10,57	10,92	10,76
DO out (%)	104,0	81,5	91,6	88,9	100,6	88,6	88,2	86,3	90,3	86,6
DO out (mg L ⁻¹)	10,21	8,18	9,19	8,80	10,00	8,73	8,70	8,59	9,01	8,58
Total Biomass (kg)	0	30,02	29,79	29,79	29,79	29,31	29,42	29,53	29,64	29,75
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	0	0,72	0,77	1,31	1,90	1,57	1,92	2,01	1,93	2,20
No. Fish	0	158	158	157	157	157	155	155	155	155
Mortality (%)	0	0	0,63	0,63	0,63	1,91	1,91	1,91	1,91	1,91
No. Dead Fish	0	0	1	0	0	2	0	0	0	0
Weight Dead Fish (kg)	0	0	0,234	0	0	0,583	0	0	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0,110	0,110	0,110	0,110	0,110	0,110

Table 3: Daily measurements in the LRS tank No. 1 between days 10 - 19.

Days	10	11	12	13	14	15	16	17	18	19
Date	4.2.2008	5.2.2008	6.2.2008	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	10,6	10,6	10,0	10,2	10,2	10,5	10,4	10,5	10,0	10,4
pН	7,57	7,54	7,55	7,54	7,57	7,52	7,57	7,61	7,57	7,56
Salinity (ppt)	20	20	21	21	21	21	20	20	21	21
DO in (%)	117,3	108,8	109,7	105,6	107,2	107,1	105,4	105,5	106,5	106,1
DO in (mg L ⁻¹)	11,63	10,76	11,01	10,53	10,68	10,65	10,47	10,51	10,66	10,55
DO out (%)	96,2	85,3	86,8	81,7	81,8	76,9	81,0	80,0	82,5	74,8
DO out (mg L ⁻¹)	9,52	8,43	8,71	8,14	8,15	7,58	8,04	7,91	8,27	7,42
Total Biomass (kg)	29,59	29,70	29,81	29,92	30,03	30,14	30,25	30,36	30,47	30,58
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	2,14	2,35	2,31	2,40	2,53	3,06	2,41	2,57	2,35	3,07
No. Fish	155	154	154	154	154	154	154	154	154	154
Mortality (%)	2,55	2,55	2,55	2,55	2,55	2,55	2,55	2,55	2,55	2,55
No. Dead Fish	1	0	0	0	0	0	0	0	0	0
Weight Dead Fish (kg)	0,275	0	0	0	0	0	0	0	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,110	0,110	0,110	0,110	0,110	0,110	0,110	0,113	0,113	0,113

Table 4: Daily measurements in the LRS tank No. 2 between days 0-9.

Days	0	1	2	3	4	5	6	7	8	9
Date	21.1.2008	22.1.2008	23.1.2008	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008
Temperature (°C)	9,9	9,5	9,9	9,9	9,9	10,5	10,3	10,2	10,1	10,6
pН	7,97	7,40	7,53	7,58	7,58	7,57	7,64	7,53	7,49	7,46
Salinity (ppt)	20	20	20	20	20	20	20	20	21	20
DO in (%)	104,2	88,6	99,3	102,4	120,0	101,8	106,6	106,2	109,7	108,8
DO in (mg L ⁻¹)	10,20	8,90	9,95	10,10	11,89	10,26	10,58	10,57	10,92	10,76
DO out (%)	104,0	80,3	92,1	89,1	100,4	88,0	88,5	86,9	88,8	84,8
DO out (mg L ⁻¹)	10,21	8,07	9,27	8,87	9,98	8,70	8,79	8,63	8,85	8,38
Total Biomass (kg)	0	30,03	29,49	29,26	29,26	28,80	28,91	28,76	28,87	28,98
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	0	0,83	0,69	1,26	1,96	1,62	1,86	2,02	2,15	2,46
No. Fish	0	158	158	156	155	155	153	153	152	152
Mortality (%)	0	0	1,27	1,91	1,91	3,20	3,20	3,85	3,85	3,85
No. Dead Fish	0	0	2	1	0	2	0	1	0	0
Weight Dead Fish (kg)	0	0	0,543	0,223	0	0,571	0	0,268	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0,110	0,110	0,110	0,110	0,110	0,110

Table 5: Daily measurements in the LRS tank No. 2 between days 10 - 19.

Days	10	11	12	13	14	15	16	17	18	19
Date	4.2.2008	5.2.2008	6.2.2008	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	10,6	10,6	10,0	10,2	10,2	10,5	10,4	10,5	10,0	10,4
pН	7,57	7,53	7,56	7,55	7,60	7,51	7,58	7,66	7,58	7,57
Salinity (ppt)	20	20	21	21	21	21	20	20	21	21
DO in (%)	117,3	108,8	109,7	105,6	107,2	107,1	105,4	105,5	106,5	106,1
DO in (mg L ⁻¹)	11,63	10,76	11,01	10,53	10,68	10,65	10,47	10,51	10,66	10,55
DO out (%)	96,7	87,3	86,5	85,2	85,4	79,2	80,0	82,4	84,3	75,3
DO out (mg L ⁻¹)	9,56	8,64	8,67	8,49	8,52	7,86	7,94	8,28	8,46	7,47
Total Biomass (kg)	29,09	29,20	29,31	29,42	29,53	29,64	29,75	29,86	29,97	30,08
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	2,14	2,18	2,40	2,08	2,19	2,82	2,55	2,24	2,20	3,07
No. Fish	152	152	152	152	152	152	152	152	152	152
Mortality (%)	3,85	3,85	3,85	3,85	3,85	3,85	3,85	3,85	3,85	3,85
No. Dead Fish	0	0	0	0	0	0	0	0	0	0
Weight Dead Fish (kg)	0	0	0	0	0	0	0	0	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,110	0,110	0,110	0,110	0,110	0,110	0,110	0,113	0,113	0,113

Table 6: Daily measurements in the new water inlet to LRS between days 0-9.

Days	0	1	2	3	4	5	6	7	8	9
Date	21.1.2008	22.1.2008	23.1.2008	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008
Temperature (°C)	9,9	9,9	9,8	9,5	9,2	9,7	9,5	9,4	9,4	9,5
pН	7,98	7,98	7,87	7,86	7,79	7,82	7,87	7,78	7,78	7,81
Salinity (ppt)	20	20	20	20	20	20	20	20	21	20
DO (%)	107,2	106,3	108,1	99,3	96,3	89,9	94,3	98,0	98,7	105,0
DO (mg L ⁻¹)	10,67	10,27	10,89	9,87	9,58	9,18	9,58	10,12	10,15	10,49
Flow rate (L min ⁻¹)	12	12	12	12	12	12	12	12	12	12
Flow rate (L min ⁻¹ kg ⁻¹)	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2

Table 7: Daily measurements in the new water inlet to LRS between days 10 - 19.

Days	10	11	12	13	14	15	16	17	18	19
Date	4.2.2008	5.2.2008	6.2.2008	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	9,0	8,8	8,4	8,8	8,6	8,7	8,8	9,0	8,5	8,7
pH	7,77	7,93	7,90	7,78	7,86	7,82	7,87	7,89	7,87	7,88
Salinity (ppt)	20	21	21	21	21	21	20	20	21	21
DO in (%)	116,8	110,2	102,4	95,2	96,1	85,3	79,2	73,5	73,1	77,6
DO in (mg L ⁻¹)	11,89	11,29	10,64	9,69	9,91	8,76	8,13	7,93	7,59	8,04
Flow rate (L min ⁻¹)	12	12	12	12	12	12	12	12	12	12
Flow rate (L min ⁻¹ kg ⁻¹)	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2

Table 8: Values of different water quality parameters calculated in LRS tank No. 1 two times per week during the experimental time and their Removal rate values.

			Da	ıys		
Items	5	8	10	13	15	18
TC (mg L ⁻¹)	72,98	74,38	60,18	55,03	53,52	87,23
CO ₂ (mg L ⁻¹)	3,34	3,97	2,90	2,38	2,99	3,37
Removal Rate CO ₂ (mgCO ₂ min ⁻¹ kg ⁻¹)	1,16	1,26	1,07	0,58	1,47	1,36
Removal Rate CO ₂ (%)	116	126	107	58	147	136
TAN (mg L ⁻¹)	0,181	0,164	0,171	0,359	0,383	0,496
Removal Rate TAN (mgTAN min ⁻¹ kg ⁻¹)	0,020	0,016	0,008	0,028	0,035	0,049
Removal Rate TAN (%)	2,0	1,6	0,8	2,8	3,5	4,9
NH ₃ -N (mg L ⁻¹)	0,001	0,001	0,001	0,002	0,003	0,003
TSS (mg L ⁻¹)	-	1,06	1,24	2,15	3,55	5,55
Removal Rate TSS (mgTSS min ⁻¹ kg ⁻¹)	-	0,97	0,96	1,03	1,00	1,06
Removal Rate TSS (%)	-	97	96	103	100	106

Table 9: Values of different water quality parameters calculated in LRS tank No. 2 two times per week during the experimental time and their Removal rate values.

			Da	ıys		
Items	5	8	10	13	15	18
TC (mg L ⁻¹)	72,99	74,38	63,62	58,47	50,60	86,20
CO ₂ (mg L ⁻¹)	3,39	3,97	3,38	2,91	2,83	3,09
Removal Rate CO ₂ (mgCO ₂ min ⁻¹ kg ⁻¹)	1,23	1,29	1,59	1,14	1,33	1,11
Removal Rate CO ₂ (%)	123	129	159	114	133	111
TAN (mg L ⁻¹)	0,171	0,163	0,168	0,343	0,368	0,468
Removal Rate TAN (mgTAN min ⁻¹ kg ⁻¹)	0,011	0,014	0,005	0,012	0,021	0,021
Removal Rate TAN (%)	1,1	1,4	0,5	1,2	2,1	2,1
NH ₃ -N (mg L ⁻¹)	0,001	0,001	0,001	0,003	0,003	0,003
TSS (mg L ⁻¹)	-	1,02	1,23	2,10	3,59	5,60
Removal Rate TSS (mgTSS min ⁻¹ kg ⁻¹)	-	0,96	0,97	1,00	1,05	1,13
Removal Rate TSS (%)	-	96	97	100	105	113

Table 10: Values of different water quality parameters calculated in the water inlet tanks of the LRS two times per week during the experimental time and the water flow using inside the tanks in the system.

		Days									
Items	5	8	10	13	15	18					
TC (mg L ⁻¹)	73,70	76,04	59,69	58,03	50,07	90,98					
CO ₂ (mg L ⁻¹)	2,21	2,72	1,84	1,80	1,51	1,98					
TAN (mg L ⁻¹)	0,161	0,149	0,163	0,331	0,347	0,447					
NH ₃ -N (mg L ⁻¹)	0,002	0,001	0,002	0,004	0,004	0,005					
TSS (mg L ⁻¹)	-	0,10	0,29	1,12	2,55	4,47					
Water flow (L min ⁻¹)	30	30	30	30	30	30					

Table 11: Values of different water quality parameters calculated in the new water inlet to LRS two times per week during the experimental time and the water flow using within the system.

		Days								
Items	5	8	10	13	15	18				
TC (mg L ⁻¹)	73,98	73,54	60,05	58,22	51,18	92,67				
CO ₂ (mg L ⁻¹)	1,91	2,16	1,29	1,32	1,39	1,96				
TAN (mg L ⁻¹)	0,002	0	0,002	0,002	0	0				
NH ₃ -N (mg L ⁻¹)	0	0	0	0	0	0				
TSS (mg L ⁻¹)	-	0,15	0,20	0,20	0,10	0,15				
Water flow (L min ⁻¹)	12	12	12	12	12	12				

Tables of Measurements for the Recirculating Aquaculture System (RAS)

Table 12: Daily measurements in the RAS tank No. 1 between days 0 - 9.

Days	0	1	2	3	4	5	6	7	8	9
Date	21.1.2008	22.1.2008	23.1.2008	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008
Temperature (°C)	9,0	8,9	10,5	10,5	10,6	12,3	12,0	13,4	12,4	13,0
pН	8,01	7,43	7,56	7,57	7,60	7,49	7,55	7,45	7,46	7,45
Salinity (ppt)	20	20	19	19	19	19	22	22	20	20
DO in (%)	101,8	101,2	98,9	100,2	102,0	114,7	109,5	111,3	114,8	115,6
DO in (mg L ⁻¹)	10,40	10,36	9,77	9,80	9,97	10,95	10,80	10,63	10,71	10,82
DO out (%)	101,7	95,3	92,5	88,3	86,1	87,2	93,2	85,9	89,7	88,2
DO out (mg L ⁻¹)	10,40	9,61	9,17	8,62	8,57	8,41	8,93	8,02	8,54	8,26
Total Biomass (kg)	0	30,02	29,45	29,26	29,05	29,05	29,08	28,86	28,89	28,93
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	0	0,75	0,61	1,21	1,45	2,62	1,93	2,71	2,25	2,66
No. Fish	0	158	158	156	155	154	154	154	153	153
Mortality (%)	0	0,00	1,27	1,91	2,55	2,55	2,55	3,20	3,20	3,20
No. Dead Fish	0	0	2	1	1	0	0	1	0	0
Weight Dead Fish (kg)	0	0	0,567	0,198	0,207	0	0	0,263	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0	0,035	0,035	0,035	0,035	0,035

Table 13: Daily measurements in the RAS tank No. 1 between days 10 - 19.

Days	10	11	12	13	14	15	16	17	18	19
Date	4.2.2008	5.2.2008	6.2.2008	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	13,6	13,5	13,8	14,2	12,4	12,3	12,8	11,3	11,2	11,4
pН	7,50	7,55	7,61	7,58	7,67	7,61	7,70	7,80	7,71	7,64
Salinity (ppt)	19	19	19	20	20	21	20	20	21	21
DO in (%)	118,5	113,0	114,7	111,7	114,2	114,0	112,5	111,7	114,2	112,8
DO in (mg L ⁻¹)	11,08	10,50	10,59	10,19	10,87	10,84	10,60	10,90	11,15	10,96
DO out (%)	104,3	90,0	91,5	93,1	98,9	93,7	97,3	98,8	94,6	89,1
DO out (mg L ⁻¹)	9,68	8,37	8,44	8,52	9,40	8,91	9,18	9,56	9,23	8,68
Total Biomass (kg)	28,96	29,00	29,03	29,07	29,10	29,14	29,17	29,21	29,25	29,29
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	1,45	2,20	2,22	1,72	1,52	1,99	1,46	1,38	1,97	2,34
No. Fish	153	153	153	153	153	153	153	153	153	153
Mortality (%)	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20
No. Dead Fish	0	0	0	0	0	0	0	0	0	0
Weight Dead Fish (kg)	0	0	0	0	0	0	0	0	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,040	0,040	0,040

Table 14: Daily measurements in the RAS tank No. 2 between days 0 - 9.

Days	0	1	2	3	4	5	6	7	8	9
Date	21.1.2008	22.1.2008	23.1.2008	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008
Temperature (°C)	9,0	8,9	10,6	10,5	10,6	12,3	12,0	13,4	12,4	13,0
pН	8,01	7,43	7,56	7,56	7,61	7,50	7,54	7,48	7,48	7,45
Salinity (ppt)	20	20	19	19	19	19	22	22	20	20
DO in (%)	101,8	101,2	98,9	100,2	102,0	114,7	109,5	111,3	114,8	115,6
DO in (mg L ⁻¹)	10,40	10,36	9,77	9,80	9,97	10,95	10,80	10,63	10,71	10,82
DO out (%)	101,7	97,6	91,8	88,6	85,5	87,4	94,3	87,5	90,4	91,2
DO out (mg L ⁻¹)	10,40	9,95	9,11	8,67	8,49	8,42	9,02	8,16	8,62	8,68
Total Biomass (kg)	0	30,17	29,71	29,71	29,71	29,15	29,19	29,22	29,26	29,03
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	0	0,41	0,67	1,14	1,49	2,60	1,83	2,54	2,14	2,21
No. Fish	0	158	158	156	156	156	154	154	154	154
Mortality (%)	0	0	1,27	1,27	1,27	2,55	2,55	2,55	2,55	3,20
No. Dead Fish	0	0	2	0	0	2	0	0	0	1
Weight Dead Fish (kg)	0	0	0,457	0	0	0,563	0	0	0	0,260
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0	0,035	0,035	0,035	0,035	0,035

Table 15: Daily measurements in the RAS tank No. 2 between days 10 - 19.

Days	10	11	12	13	14	15	16	17	18	19
Date	4.2.2008	5.2.2008	6.2.2008	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	13,6	13,5	13,8	14,2	12,4	12,3	12,8	11,3	11,2	11,4
pН	7,51	7,56	7,60	7,57	7,67	7,61	7,69	7,80	7,72	7,65
Salinity (ppt)	19	19	19	20	20	21	20	20	21	21
DO in (%)	118,5	113,0	114,7	111,7	114,2	114,0	112,5	111,7	114,2	112,8
DO in (mg L ⁻¹)	11,08	10,50	10,59	10,19	10,87	10,84	10,60	10,90	11,15	10,96
DO out (%)	101,4	92,3	90,3	90,7	96,2	93,0	96,8	95,3	96,7	92,2
DO out (mg L ⁻¹)	9,38	8,57	8,33	8,30	9,14	8,85	9,11	9,27	9,43	8,90
Total Biomass (kg)	29,07	29,10	29,14	29,17	29,21	29,24	29,13	29,17	29,21	29,25
MO ₂ (mgO ₂ min ⁻¹ kg ⁻¹)	1,75	1,99	2,33	1,94	1,78	2,04	1,53	1,68	1,77	2,11
No. Fish	153	153	153	153	153	153	153	152	152	152
Mortality (%)	3,20	3,20	3,20	3,20	3,20	3,20	3,85	3,85	3,85	3,85
No. Dead Fish	0	0	0	0	0	0	1	0	0	0
Weight Dead Fish (kg)	0	0	0	0	0	0	0,142	0	0	0
Flow rate (L min ⁻¹)	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,040	0,040	0,040

Table 16: Daily measurements in the new water inlet to the RAS between days 0-9.

Days	0	1	2	3	4	5	6	7	8	9
Date	21.1.2008	22.1.2008	23.1.2008	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008
Temperature (°C)	8,9	9,4	10,3	10,2	11,6	11,6	11,6	10,7	9,0	8,8
рН	8,01	7,96	7,88	7,85	7,80	7,79	7,81	7,95	7,76	7,87
Salinity (ppt)	20	20	19	19	19	19	22	22	20	20
DO (%)	109,3	108,9	108,4	106,9	96,5	101,6	101,9	102,8	108,0	99,7
DO (mg L ⁻¹)	11,12	11,02	10,99	10,78	9,54	9,87	10,03	10,89	11,02	10,31
Flow rate (L min ⁻¹)	12	12	12	12	5	5	3	3	3	3
Flow rate (L min ⁻¹ kg ⁻¹)	0.08	0.08	0.08	0.08	0,08	0,08	0,05	0,05	0,05	0,05

Table 17: Daily measurements in the new water inlet to the RAS between days 10 - 19.

Days	10	11	12	13	14	15	16	17	18	19
Date	4.2.2008	5.2.2008	6.2.2008	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	8,7	8,3	6,6	8,6	7,2	6,6	8,6	5,2	5,4	5,3
рН	7,91	7,94	7,87	7,93	7,99	7,87	8,33	7,89	7,89	7,90
Salinity (ppt)	19	19	19	20	20	21	20	20	21	21
DO (%)	119,2	109,0	102,1	99,6	95,9	92,1	93,1	74,6	73,6	81,4
DO (mg L ⁻¹)	12,23	11,36	11,04	10,63	10,31	10,05	9,99	8,37	8,23	9,15
Flow rate (L min ⁻¹)	3	3	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Flow rate (L min ⁻¹ kg ⁻¹)	0,05	0,05	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008

Table 18: Daily measurements in the outlet water from the biofilter in the RAS between days 3 - 12.

Days	3	4	5	6	7	8	9	10	11	12
Date	24.1.2008	25.1.2008	28.1.2008	29.1.2008	30.1.2008	31.1.2008	1.2.2008	4.2.2008	5.2.2008	6.2.2008
Temperature (°C)	10,5	10,8	12,3	12,0	13,0	12,3	13,0	13,5	13,5	13,8
pН	7,42	7,45	7,59	7,66	7,63	7,73	7,48	7,59	7,60	7,63
DO (%)	97,1	97,3	96,9	97,9	97,0	98,5	97,0	102,0	95,1	95,4
DO (mg L ⁻¹)	9,30	9,35	9,23	9,43	9,09	9,38	9,10	9,73	8,83	8,79

Table 19: Daily measurements in the outlet water from the biofilter in the RAS between days 13 - 19.

Days	13	14	15	16	17	18	19
Date	7.2.2008	8.2.2008	11.2.2008	12.2.2008	13.2.2008	14.2.2008	15.2.2008
Temperature (°C)	14,2	12,3	12,2	12,8	11,2	11,0	11,3
рН	7,66	7,73	7,71	7,73	7,80	7,78	7,80
DO (%)	94,7	96,8	97,1	96,3	96,1	96,2	95,1
DO (mg L ⁻¹)	8,66	9,21	9,25	9,12	9,38	9,40	9,28

Table 20: Values of different water quality parameters calculated in RAS tank No. 1 two times per week during the experimental time and their Removal rate values.

	Days					
Items	5	8	10	13	15	18
TC (mg L ⁻¹)	83,49	75,74	67,08	68,93	66,89	97,79
CO ₂ (mg L ⁻¹)	3,91	4,43	3,66	2,84	2,03	1,80
Removal Rate CO ₂ (mgCO ₂ min ⁻¹ kg ⁻¹)	1,54	2,02	1,83	1,06	0,75	0,76
Removal Rate CO ₂ (%)	154	202	183	106	75	76
TAN (mg L ⁻¹)	0,251	0,779	0,890	1,369	1,483	1,511
Removal Rate TAN (mgTAN min ⁻¹ kg ⁻¹)	0,006	0,047	0,013	-0,049	-0,055	-0,068
Removal Rate TAN (%)	0,6	4,7	1,3	-4,9	-5,5	-6,8
NH ₃ -N (mg L ⁻¹)	0,001	0,004	0,005	0,012	0,014	0,014
TSS (mg L ⁻¹)	-	0,90	1,55	2,30	5,25	8,85
Removal Rate TSS (mgTSS min ⁻¹ kg ⁻¹)	-	0,21	0,18	0,15	0,10	0,10
Removal Rate TSS (%)	-	21	18	15	10	10

Table 21: Values of different water quality parameters calculated in RAS tank No. 2 two times per week during the experimental time and their Removal rate values.

	Days					
Items	5	8	10	13	15	18
TC (mg L ⁻¹)	83,86	75,49	68,07	68,24	66,98	92,54
CO ₂ (mg L ⁻¹)	3,67	4,22	3,63	2,99	2,09	1,93
Removal Rate CO ₂ (mgCO ₂ min ⁻¹ kg ⁻¹)	1,29	1,77	1,79	1,20	0,82	0,71
Removal Rate CO ₂ (%)	129	177	179	120	82	71
TAN (mg L ⁻¹)	0,251	0,790	0,893	1,378	1,494	1,529
Removal Rate TAN (mgTAN min ⁻¹ kg ⁻¹)	0,005	0,058	0,016	-0,039	-0,044	-0,050
Removal Rate TAN (%)	0,5	5,8	1,6	-3,9	-4,4	-5,0
NH ₃ -N (mg L ⁻¹)	0,001	0,004	0,005	0,012	0,014	0,014
TSS (mg L ⁻¹)	-	0,95	1,56	2,32	5,24	8,85
Removal Rate TSS (mgTSS min ⁻¹ kg ⁻¹)	-	0,26	0,19	0,17	0,09	0,10
Removal Rate TSS (%)	-	26	19	17	9	10

Table 22: Values of different water quality parameters calculated in the water inlet tanks of the RAS two times per week during the experimental time.

	Days							
Items	5	8	10	13	15	18		
TIC (mg L ⁻¹)	86,04	74,32	66,57	70,82	69,65	92,56		
CO ₂ (mg L ⁻¹)	2,42	2,49	1,90	1,82	1,30	1,24		
TAN (mg L ⁻¹)	0,246	0,734	0,877	1,416	1,537	1,577		
NH ₃ -N (mg L ⁻¹)	0,003	0,008	0,010	0,020	0,022	0,023		
TSS (mg L ⁻¹)	-	0,70	1,38	2,15	5,15	8,75		
Water Flow (L min ⁻¹)	30	30	30	30	30	30		

Table 23: Values of different water quality parameters calculated in the new water inlet to the RAS two times per week during the experimental time.

	Days						
Items	5	8	10	13	15	18	
TIC (mg L ⁻¹)	87,09	73,12	65,96	70,34	69,42	92,98	
CO ₂ (mg L ⁻¹)	1,79	1,92	1,68	1,81	1,12	1,24	
TAN (mg L ⁻¹)	0,003	0	0,004	0,001	0,001	0	
NH ₃ -N (mg L ⁻¹)	0	0	0	0	0	0	
TSS (mg L ⁻¹)	-	0,15	0,20	0,20	0,20	0,15	
Flow rate (L min ⁻¹)	5	3	3	0,5	0,5	0,5	
Flow rate (L min ⁻¹ kg ⁻¹)	0.08	0.05	0.05	0.008	0.008	0.008	

Table 24: Values of different water quality parameters calculated in the outlet water from the biofilter in the RAS two times per week during the experimental time.

	Days					
Items	5	8	10	13	15	18
TIC (mg L ⁻¹)	86,04	77,90	71,01	65,79	65,52	93,72
CO ₂ (mg L ⁻¹)	2,42	3,54	2,94	2,67	2,42	2,87
TAN (mg L ⁻¹)	0,240	0,724	0,868	1,449	1,556	1,652
Removal Rate TAN (mgTAN min ⁻¹ kg ⁻¹)	0,012	0,060	0,026	-0,074	-0,066	-0,131
Removal Rate TAN (%)	1,2	6,0	2,6	-7,4	-6,6	-13,1
NH ₃ -N (mg L ⁻¹)	0,002	0,008	0,006	0,014	0,015	0,018
NO ₂ -N (mg L ⁻¹)	0	0	0,22	0,44	0,748	1,10
NO ₃ -N (mg L ⁻¹)	0	0	0	0,099	0,33	0,66
TSS (mg L ⁻¹)	-	0,50	0,75	1,30	4,05	8,00

Final report Internship

Research on growth of Arctic Charr and water quality in water re-use systems at different water exchange rates



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Table of contents

1. Introduction	3
1.1 Background information	3
1.2 Water quality and growth experiment	3
1.2.1 Water quality	4
1.2.2 Growth	4
1.3 Objectives	4
1.4 Hypotheses	4
2. Materials and Methods	5
2.1 System Characteristics	5
2.1.1 Pilot scale recirculation systems	5
2.1.2 Inlet water characteristics	8
2.1.3 Exchange rate and recirculation rate	8
2.1.4 Fish characteristics, start values	9
2.2 Maintainance	10
2.2.1 Feeding	10
2.2.2 Cleaning procedure	10
2.2.3 Regulating exchange rate	11
2.2.4 Regulating temperature	11
2.3 Measurements and Analyses	11
2.3.1 Suspended Solids	12
2.3.2 Total Ammonium Nitrogen concentration (TAN)	12
2.3.3 Carbon Dioxide (CO ₂)	12
2.3.4 pH and Total Alkalinity (TA)	12
3. Results	13
3.1 Water quality	13
'Compare water quality between the three systems'	13
3.1.1 Total Ammonia Nitrogen (TAN)	13
3.1.2 Suspended Solids	15
3.1.3 Temperature	16
3.1.4 Oxygen	17
3.1.5 pH	19
3.2 Growth	19
'Compare the growth rate of the Arctic Char between the different systems'	19
4. Discussion	20
5. Conclusions	24

1. Introduction

1.1 Background information

About fifty percent of the Icelandic export is based on fish or fish products [Icelandic Ministry of Fisheries and Agriculture]. With those fifty percent it is by far the most important industrial business in Iceland.

However, on many land based fish farms in Iceland the cold and geothermal water has to be pumped from wells to the fish farm. The costs of pumping the water can be reduced by reusing water in the station. Before the water can be reused it has to be filtrated to reduce the amount of ammonium and suspended solids. Another important process is to saturate the water with oxygen.

When the water is reused there is a risk factor attached to it. Even though the water is filtrated and saturated with oxygen, there can be accumulation of suspended solids, ammonium, CO2 and an increasing temperature depending on the water exchange rate. There is not a lot of knowledge about how these factors have influence on the growth of Arctic Char. In order to explain if the water exchange rate in a system has a substantial effect on water quality and the growth of fish an experiment is performed.

The experiment is performed at research station Verið in Sauðarkrokur, Iceland. For the experiment three different systems are used with each a different water exchange rate to compare the water quality and growth of Arctic Char.

The experiment is executed by two bachelor students Environmental Science of the University of Applied Sciences HAS Den Bosch, the Netherlands, under supervision of H. Thorarensen Ph.D (head of the department of Aquaculture and Fishbiology of Hólar University College Iceland) and R. Jóhannsson Ph.D (head of the division of Genetics and Aquaculture of Matís, Iceland). The process of the internship is under supervision of MSc. A. van de Koolwijk (HAS Den Bosch, University of Applied Sciences, the Netherlands)

1.2 Water quality and growth experiment

To compare different water purification methods three systems are used. In these systems different purification components are applied. In the beginning of the experiment, the water flows in the systems are set equal. Only the water exchange rate is different in each system and is monitored and adjusted daily depending on the weight of the fish in the systems. Daily the water quality and quantity parameters are monitored. Once a week the total ammonium nitrogen (TAN), suspended solids and growth are measured. Before putting the Arctic Char into the system, the different components in the systems are optimized and adjusted. The experiment is mostly based on the water quality but the growth is also monitored to see the influence of different water quality in relation with the growth.

1.2.1 Water quality

To monitor the water quality the measurements are performed daily. This includes daily measurements of temperature in °C, oxygen level in [%], pH and the water exchange rate (water quantity).

Once a week suspended solids in [mg/l], total ammonium nitrogen (TAN) in [mg/l] and CO2 in [%] are measured. The samples for the measurements are taken from each individual tank, reservoir, inlet water, biofilter in and out. The samples are taken every day at the same time and before feeding the fish. In this way, the whole system is analyzed on all the different components in the water.

1.2.2 Growth

The growth of the fish is measured weekly. The results from this measurement are used to calculate the water exchange rate for each system. The weight of the fish in the tanks at the beginning of the experiment is the same in each system and the amount of feed is the same for each tank.

1.3 Objectives

In this experiment, there are two main objectives. Objective one is to compare the different water purification methods and different water exchange rates in relation to the water quality. The second objective is to monitor the effect of different water exchange rates or water quality on the growth of the fish.

- 1. Compare water quality between the three systems.
- 2. Compare the growth rate of the Arctic Char between at different water exchange rates.

1.4 Hypotheses

There are two hypotheses formulated, one for each objective.

1. 'Compare water quality between the three systems'

Hypothesis:

The expectation is that the system with the highest water exchange rate has the best water quality to grow Arctic Char because there is a lot of fresh water coming in without pollutions like ammonium or suspended solids and with a relatively high amount of oxygen in it.

Compare the growth rate of the Arctic Char at different water exchange rates

Hypothesis:

The expectation is that system three, the one with the highest water exchange rate, has the highest growth rate. The due to an high concentration of oxygen and the expected relatively low amount of ammonium and other contaminants.

2. Materials and Methods

This chapter describes the method and materials used for the experiment. In paragraph 2.1 the used systems, inlet water and fish characteristics are described. Paragraph 2.2 explains how the systems and fish were maintained during the experiment. Paragraph 2.3 describes what measurements and analysis were performed to monitor water quality and growth. The experiment was conducted over 40 days, between 4-5-2009 and 12-6-2009.

2.1 System Characteristics

2.1.1 Pilot scale recirculation systems

In this experiment the water quality and growth of fish are compared at different water exchange rates in pilot scale recirculation production systems. As showed in figures 2.1-2.3 all systems in general comprise 2 tanks (0,7m³ each), a reservoir with overflow, an aerator (enriched with liquid oxygen) and a separator for suspended solids.

In system 2, the system with the lowest water exchange rate (paragraph 2.1.3), two modifications were made to prevent a lethal accumulation of suspended and dissolved substances. A bio filter was installed, filtering water from the reservoir in order to remove ammonia. Adjacent to this, a tube settler was installed to filter suspended solids from the recirculating water. All water from tanks 3 and 4 flows through the tube settler and is directly guided to the reservoir, through which no overflow separator or overflow (as in system 1 and 3) is used to prevent suspended solids from recirculating.

The recirculation rate is kept equal in all systems at 70L/min. The flow rate through the aerator is set between 81-100L/min. The flow rate through the bio filter is 106L/min. The flow rate of the inlet water and flow rate through the overflow in the reservoir are considered equal within every system (in = out). The flow rate is not described in figures 2.1-2.3, because the flowe rate changed during the experiment to maintain the desired water exchange rate in the systems (paragraph 2.1.3).

Figure 2.1

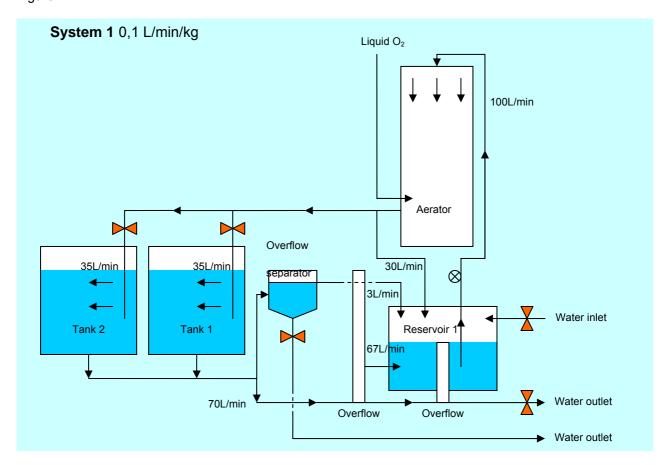


Figure 2.2

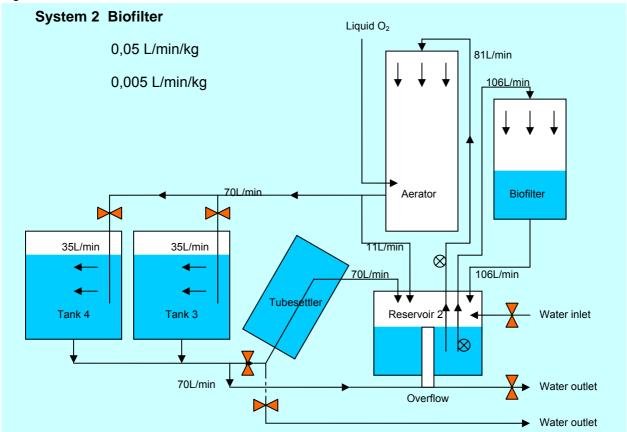
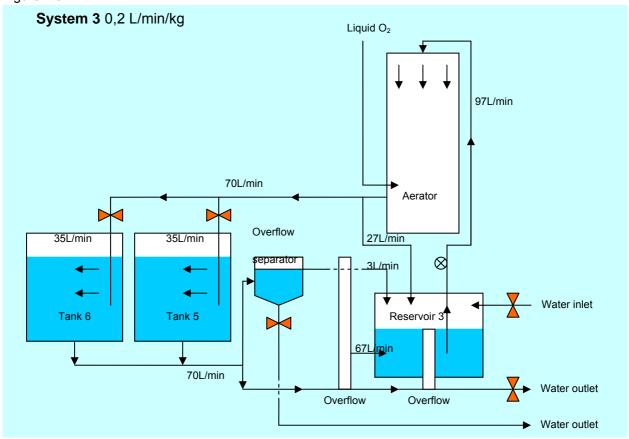


Figure 2.3



2.1.2 Inlet water characteristics

Freshwater is used for all three systems, Salinity for all systems is therefore 0.

Water pressure in the piping before entering the systems (at the water inlet) is considered constant. The temperature of the inlet water may fluctuate due to a changing temperature at the extraction point or within the piping.

2.1.3 Exchange rate and recirculation rate

Three recirculation systems are used with each a different water exchange rate. The recirculation rate (flow through the aerator and both tanks) is equal for all three systems.

Table 2.1 presents the exchange rate (L/min/kg) and recirculation rate (L/min) of each system in the first three weeks of the experiment. The recirculation rate is the sum of the recirculation of the two tanks per system and equal to the flow through the aerator.

Table 2.1 Exchange rate and recirculation rate in each system at the start of the experiment.

System	Exchange rate (L/min/kg)	Recirculation rate (L/min)
1	0,1	70
2	0,05	70
3	0,2	70

In the fourth week (day 22) the exchange rate of system 2 was lowered tenfold to 0,005 L·min⁻¹·kg⁻¹, as showed in table 2.2, to increase the Total Ammonium Nitrogen (TAN) concentration in order to the start the biofilter.

Table 2.2 Exchange rate and recirculation rate in each system in the fourth week

System	Exchange rate (L·min ⁻¹ ·kg ⁻¹)	Recirculation rate (L·min ⁻¹)
1	0,1	70
2	0,005	70
3	0,2	70

In order to maintain the exchange rate during the experiment, the growth of the fish is measured every week and the amount of inlet water adjusted every day.

2.1.4 Fish characteristics, start values

Each system was started with an equal loading of circa 15kg Arctic Charr. Table 2.3 presents a summary of the amount of individuals, average weight and total weight per system. Table 2.4 presents the same characteristics for each tank.

Table 2.3 Total weight of fish per system, amount of individuals and average weight of fish per system.

System	Weight _{system} (gram)	Individuals	Average weight (gram)
1	15.000	237	63
2	15.010	227	66
3	14.970	248	60

Table 2.4 Total weight of fish per tank, amount of individuals and average weight of fish per tank.

System	Tank	Weight _{tank} (gram)	Individuals	Average weight (gram)
1	1	7.515	121	62
I	2	7.485	116	65
2	3	7.520	114	66
2	4	7.490	113	66
3	5	7.485	120	62
3	6	7.485	128	58

The fish density in each system is defined as kg fish per m³ and is calculated with the following equation [Timmons et al., 2001]:

$$D_{fish} = m_{fish}/V_{tankvlume}$$

Deducted from this equation at the start of the experiment all systems have a fish density D_{fish} of 15,0/1,4 = 10,7 kg/m³.

2.2 Maintainance

2.2.1 Feeding

The fish were fed 6 days a week, from Monday until Saturday, during a period of 6 weeks with 3mm pellets (Table 2.5). The fish were not fed on Sunday as the weighing took place on Monday (Table 2.6). Feeding took place 2 times a day, after measurements and taking water samples. Table 2.4 presents the feeding schedule.

Table 2.4 Feeding schedule per system.

Week	Feed (g/day/system)	Feed total (g/system)
1	200	1.200
2	250	1.500
3	300	1.800
4	300	1.800
5	350	2.100
6	400	2.000*
	Total	10.400

^{*} fish not fed on Thursday because of weighing on Friday

Table 2.5 Nutrient content 3mm pellets.

Contents	Àmmount	
Dry matter	91 %	
Protein	50 %	
Fat	23 %	
Carbohydrate	9 %	
Ash	7,5 %	
Coloring agent	5 mg/kg	
Energy _{usable}	19,9 MJ/kg	
Energy _{total}	22,4 MJ/kg	

2.2.2 Cleaning procedure

Systems 1 and 3 were cleaned three times a week (Monday, Wednesday and Friday). The cleaning process includes flushing the drain of the tanks and reservoir, removing clotted suspended solids from the reservoir and cleaning the overflow separator.

System 2 was cleaned only on Fridays, given that there is no overflow separator present and nearly all suspended solids accumulate in the tube settler which, because of a relatively large volume, rarely becomes blocked.

Table 2.6 Compartments cleaned per system

System	Drain tanks	Drain reservoir	Clotted ss	Overflow separator	Tube settler
1	Х	Х	Х	Х	
2					Х
3	Х	Х	X	X	

2.2.3 Regulating exchange rate

The exchange rate was checked for all three system every day by measuring the water flow at the water inlet. The water flow was measured during 1 minute and, if necessary changed to the correct set point by opening or closing the valve. The set point for the water flow (L·min⁻¹) was calculated with the equation below. The values for the exchange rate (L·min⁻¹·kg⁻¹) are found in table 2.1 and table 2.2. The weight is determined every week by weighing all the fish per tank.

 $Q_{\text{waterflow}} = \text{exchangerate} * m_{\text{fish per system}}$

2.2.4 Regulating temperature

The temperature was measured every day and maintained between 11-12°C. For adjusting the temperature, the cold water flow through the cooling system in the reservoirs could be increased or decreased by opening or closing the valve of the cooling system. If the temperature needed to be increased, warm water was added through the same system.

2.3 Measurements and Analyses

For this experiment several parameters were measured and several analyses performed.

Five days a week the temperature and oxygen level was measured in every tank, reservoir and inlet water. Adjacent to this, the temperature of the sea water was measured. Once a week the fish were weighed and samples taken for the analysis of suspended solids, Total Ammonium Nitrogen (TAN) concentration, pH and CO_2 level for each tank, reservoir and inlet water. Thereby water samples are taken of both inlet- and outlet water of the biofilter. Methods for these analyses are defined in the following paragraphs. Table 2.7 presents the timetable for measurements and sampling. All measurements were performed and all samples for analysis were taken between 9am and 10am before the fish were fed.

Table 2.7 Timetable for measurements and sampling.

Parameter/analysis	Monday	Tuesday	Wednesday	Thursday	Friday
Temperature	X	X	X	X	X
O ₂ level	X	X	X	X	X
Suspended Solids				X	
TAN			Х		
pН		Х			
CO ₂ level		Х			
Weight	Х				

2.3.1 Suspended Solids

For measuring the amount of suspended solids 4L of water was taken in two 2L closed polyethylenetereftalate bottles and filtered via vacuum filtration (Büchner flask and funnel) through glass microfibre filters (Whatman, poresize 1,2µm).

Before filtration the glassfiber filter paper was dried for at least 24 hours at 105°C and weighed. After filtration the filter was dried again for at least 24 hours at 105°C and weighed to determine the amount of suspended solids.

2.3.2 Total Ammonium Nitrogen concentration (TAN)

The TAN is determined according to the Danish Standard DS 224. Enclosure 1 contains the English translation of the Danish Standard DS 224. Samples are taken in a 0,5L closed polyethylene bottle and are measured on the day of sampling with a reaction period of 3 hours. The absorption of indophenol blue is measured at 630nm.

2.3.3 Carbon Dioxide (CO₂)

For determining the CO_2 concentration the program CO2SYS.EXE was used. The program performs calculations relating to parameters of the carbon dioxide (CO_2) system in seawater and freshwater. The program uses two of the four measureable parameters of the CO_2 system [total alkalinity (TA), total inorganic CO_2 (TCO_2), pH, and either fugacity (fCO_2) or partial pressure of CO_2 (pCO_2)] to calculate the other two parameters at a set of input conditions (temperature and pressure) and a set of output conditions chosen by the user [Lewis, E., and D. W. R. Wallace, 1998].In this experiment the pH was measured and the TA was determined to calculate the other two parameters of the CO_2 system with CO2SYS.EXE. Furthermore the temperature was measured when the sample was taken. The guidelines for using the program to calculate the CO_2 concentration can be found in enclosure 2, the measurement of the pH and calculation of TA is described in the next paragraph.

2.3.4 pH and Total Alkalinity (TA)

For measuring the pH 0,2L of water was taken in a closed polyethylenetereftalate bottle. The pH was measured at 25°C in 0,1L of sample after 1 minute of mixing with a magnet stirrer and a maximum stabilisation period of 5 minutes. To determine the TA 10 mL standardized 0,001M HCl was added to the sample. After 1 minutes of mixing with a magnet stirrer and a maximum stabilisation period of 5 minutes the pH is measured again.

The TA is calculated with the following equation:

$$TA[mmol/L] = V_{acid} \cdot M_{acid} \cdot 10000-1250000 \cdot (10^{-pH}_{a}/fH)$$

Where V_{acid} is the volume of acid in mL, M_{acid} is the concentration of acid in mol/L, pH_a is the pH after adding the acid and fH is the functionality of hydrogen ions. The fH value is calculated using CO2SYS.EXE.

3. Results

3.1 Water quality

'Compare water quality between the three systems'

For the first objective, 'Compare water quality between the three systems', several parameters are used to monitor the water quality. In this paragraph the monitoring results are presented. For each parameter their important factors are discussed. Some parameters are influenced by other parameters and the following factors:

- 1. Water exchange rate
- 2. Flow rate through system
- 3. Fish load
- 4. Feed
- 5. System Components
- 6. Environment of experiment

The fish load, amount of feed, flow rate and environment are considered equal for all systems. Therefore the three systems can be compared; the only difference between the systems is the water exchange rate. System 2 has got two additional components in order to prevent lethal concentrations of TAN and suspended solids (components discussed in paragraph 2.1.1). The growth rate of the Arctic Charr in each system is discussed in paragraph 3.2.

3.1.1 Total Ammonia Nitrogen (TAN)

The ammonia level can be the limiting factor for growth in the water re-uses systems; at high concentrations it can be lethal. Therefore the TAN level is measured weekly.

In the beginning of the experiment the TAN concentrations in the three different systems were quite the same. However, after 24 days the TAN concentration started changing rapidly. The TAN concentration in system two starts rising rapidly after 24 days because there was a decrease of 90% of the water exchange through the system on day 22. In Chart 3.1 an overview is made of the TAN concentration at different water exchange rates.

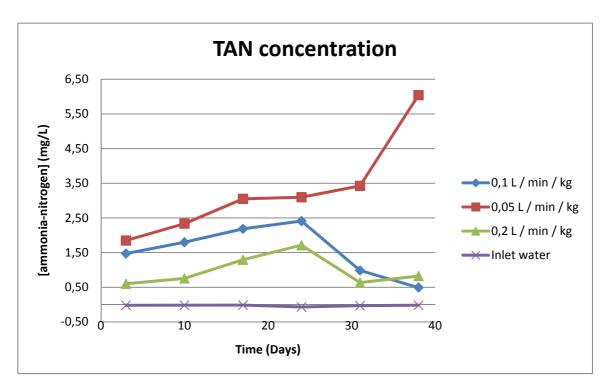


Chart 3.1 TAN concentration at different water exchange rates

Ammonia exists in two forms: unionized ammonia NH_3 , and ionized ammonia NH_4^+ . The unionized ammonia is excreted by fish, but after excretion the unionized ammonia can be converted to ionized ammonium or even to nitrite or nitrate (oxygen needed). This process primarily depends on the temperature and pH of the water. An increase of temperature and pH gives an increase of the un-ionized form of ammonia nitrogen. Chart 3.2 shows the unionized ammonia concentration at different water exchange rates.

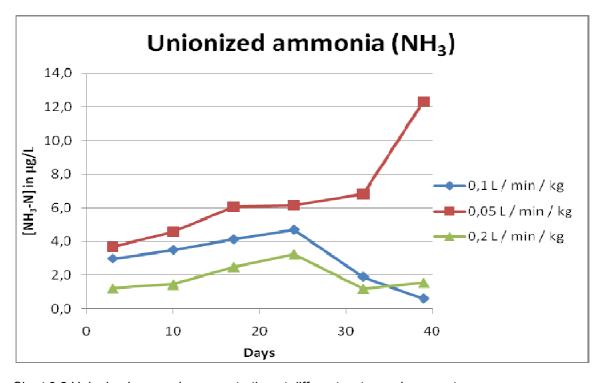


Chart 3.2 Unionized ammonia concentration at different water exchange rates

3.1.2 Suspended Solids

The amount of suspended solids is measured every week by filtrating a 4L sample trough a 0,45µm filter. As shown in Chart 3.3, the systems are stabilizing during the first two weeks. After the first two weeks the exchange rates are quite stable. On day 22 the exchange rate of system 2 has been lowered. This action had a big effect on the amount of suspended solids in system 2, the suspended solids are accumulating rapidly in the system.



Chart 3.3 Suspended solids at different water exchange rates

Figure 3.1 shows the accumulation of suspended solids in the tube settler after 1 week. The picture on the right shows the tube settler right after back flushing.



Figure 3.1 Tube settler before and after back flushing

3.1.3 Temperature

During the experiment, the water temperature was daily measured in all systems in both tanks and reservoir and inlet water. Chart 3.4 shows the average temperature of water in both tanks at different water exchange rates. The average water temperature is presented in Table 3.1.

In general, the water temperature was higher at lower water exchange rates and lower at higher water exchange rates. The average temperature at water exchange rate of 0,005L/min/kg was 13,7°C. At 0,05L/min/kg the average water temperature was 12,4°C. At 0,1L/min/kg 11,3°C and at 0,2L/min/kg 10,7°C.

The average temperature during the experiment increased except for the water temperature at 0,1L/min/kg, which was relatively stable.

At low water exchange rates (0,005, 0,05 and 0,1L/min/kg) water temperature mainly depends on environment temperature, therefore the system had to be cooled down. At higher water exchange rates (0,2L/min/kg), water temperature mainly depends on the inlet water temperature and therefore had to be warmed during this experiment due to the relatively low temperature of the inlet water.

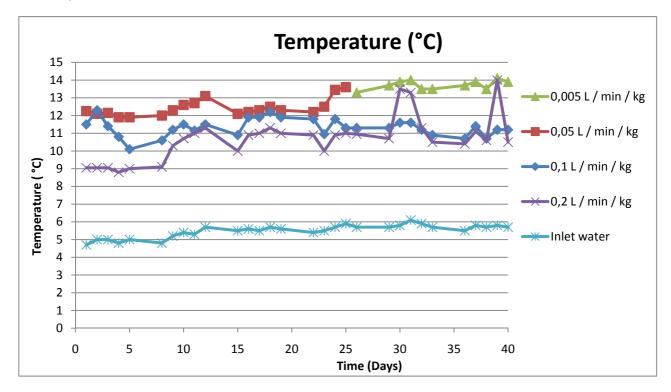


Chart 3.4 Water temperature at different water exchange rates.

Table 3.1 Average water temperature at different water exchange rates.

Exchange rate (L/min/kg)	Temperature (°C)
0,005	13,7
0,05	12,4
0,1	11,3
0,2	10,7

3.1.4 Oxygen

During the experiment, the oxygen level was measured daily in all systems in both tanks and reservoir. Chart 3.5 shows the average oxygen saturation of both tanks at different water exchange rates. The average oxygen saturation is presented in Table 3.2.

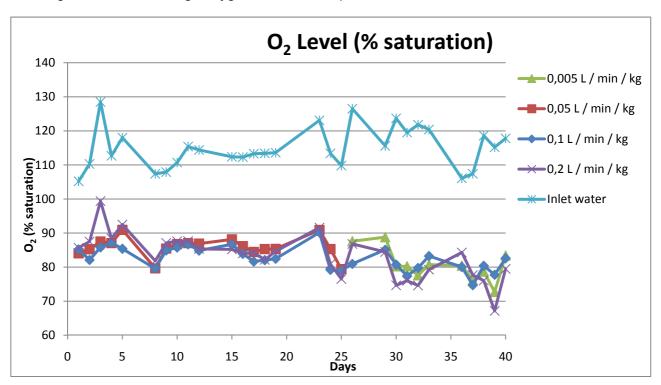


Chart 3.5 Oxygen Saturation at different water exchange rates.

There is no significant difference between the three systems in the % of oxygen saturation and dissolved oxigen. The oxygen solubility depends on the water temperature as well the salinity. As the temperature and salinity increases, the solubility decreases. [Benson and Krause, 1984]. Given that the salinity of the water is 0, the solubility can be described as:

 O_2 Solubility [mg/L] = -0,2418·Temperature + 12,014

[Equation deducted from Benson and Krause, 1984]

Given the temperature, salinity and oxygen saturation, the dissolved oxygen level is calculated. As shown in Chart 3.6, there is no significant difference in oxygen saturation between the different exchange rates. However, at relatively high water temperatures the oxygen level drops as seen at an exchange rate of 0,005L/min/kg and during day 30 and 39 at an exchange rate of 0,2L/min/kg (Chart 3.3).

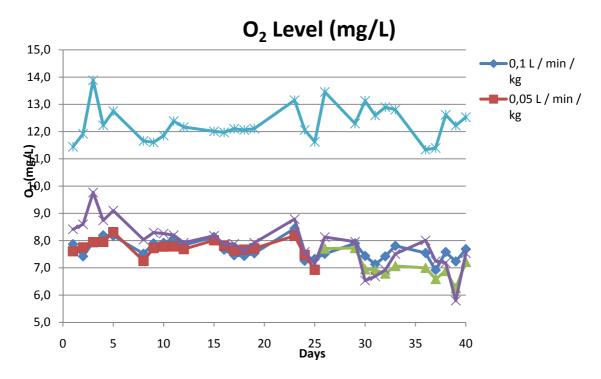


Chart 3.6 Oxygen level (mg/L) at different water exchange rates.

Table 3.2 Average Oxygen saturation and Dissolved Oxygen at different water exchange rates.

Exchange rate (L/min/kg)	Oxygen saturation (%)	Dissolved Oxygen (mg/L)
0,005	80.6	7,01
0,05	85.9	7,73
0,1	82.6	7,66
0,2	83.2	7,86