MEREVEEL PÕHINEVA KALAKASVATUSE HEITVEE PUHASTAMINE SUURVETIKATE KULTIVEERIMISE KAUDU

Euroopa Merendus ja Kalandusfondi rakenduskava 2014-2020 meetme 2.1

“Vesiviljeluse innovatsioonitoetus” projekti lõpparuanne

(projekti viitenumber 821017780003)

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2021

SISUKORD

Sissejuhatus

Suurimaks kalakasvatuse keskkonnamõjuks on kalakasvatusest üle jäävate toitainete (lämmastik ja fosfor) mõju loodusele. Kasvatuse protsessis jääb paratamatult mingi osa söödaga süsteemi sisse toodavast ainest kasutamata. Samuti satub suur osa söödas sisalduvaid toitaineid kalade väljaheidete ja muude metabolismiproduktide näol veekeskkonda, kust see tuleb siis kas kokku koguda või leida lahendus selle ohutul viisil looduskeskkonda viimiseks. Avatud kalasumpade puhul lahjendatakse kogu kalakasvatusest tulenev toitainete koormus loodusliku vee liikumisega ümbritsevasse keskkonda. Kinnistes süsteemides tuleb investeerida tehnoloogiasse, mis selle kahjuliku aine kokku korjab ja süsteemist välja viib. Lahendusi on olemas mitmesuguse erineva efektiivsuse ja maksumusega. Üheks Läänemere piirkonna jaoks perspektiivseks kalakasvatuse vormiks saaks olla poolkinnised süsteemid, kus kala kasvatataks maapealsetes kinnistes tankides kasutades läbivoolavat merevett. Sellisel lahendusel on mitmeid eeliseid nii võrreldes avatud sumpades kasvatamisega (ilmastikuolud, jääolud) kui ka täiesti kinniste maapealsete magedat vett kasutavate süsteemidega (eelkõige vee kasutamisega seotud kulud). Seni on just selliste poolkinniste, mereveel baseeruvate süsteemide rakendamine Eestis takerdunud ülemäärase toitainete koormuse riski tõttu. Hetkel on Läänemere-äärsed riigid võtnud endale kohustuse vähendada teatud fikseeritud määral oma territooriumilt tulenevat toitainete (lämmastik ja fosfor) koormust (HELCOMi Läänemere tegevuskava). Samuti on Veepoliitika Raamdirektiivi järgne rannikuvee seisundi hindamine hinnanud enamus rannikuveekogumeid „kesisesse“ seisundiklassi. Seega ei ole hetkel keskkonna järelvalvega tegelevate asutuste arvates vastuvõetav täiendava olulise toitainete koormuse lisamine merekeskkonnale. Kalakasvatuse sektori seisukohalt on ainuke väljapääs rakendada tehnoloogiaid, mis tagaks kas nullemissiooni või nullilähedase toitainete emissiooni. Traditsiooniliste filtersüsteemide kasutamine on väga kallis ja muudab investeerimise mahu tõttu ettevõtmise majanduslikult mittejätkusuutlikuks. Üheks alternatiivseks lahenduseks olekski looduslikel toitainete kasutajatel põhinevad biofiltersüsteemid.

Makrovetikatel põhinevaid kalakasvatuse jääkvee puhastamise süsteeme on maailmas rakendatud mitmel pool (näiteks Baloo et al 2014). Hiljutised uuringud näitavad üsna häid toitainete eemaldamise tulemusi ja on ka isegi saadaval kommertsplatvormil põhinevaid lahendusi, mida kalakasvatuse jaoks sisse osta. Samas kõik need lahendused põhinevad ookeanivees elavatel organismidel, kes Läänemere tingimustes ellu ei jää (Felaco 2014). Laialt kasutatakse makrovetikaid asula reovee puhastamisel (Mehta & Gaur 2005).

Käesoleva projekti eesmärgiks oli välja arendada Läänemere tingimustesse sobiv makrovetikatel põhinev veest toitainete eemaldamise tehnoloogia.

Viimase aja teadustulemustele põhinedes võib väita, et kultiveerimiseks sobivamateks Läänemere liikideks võiksid olla kiirelt arenevad niitjad rohevetikad (Felaco 2014). Mujal katsetatud liikidest on meil esinevatest kõige lähedasemad liigid perekonnast *Ulva* ja ka perekonnast *Cladophora*. Seega oli esimeses järgus kavas katsetada just liikidega *Ulva intestinalis* ja *Cladophora glomerata*. Mõlemad liigid on kiire biomassi arenguga, võivad paljuneda ka vegetatiivselt, võivad areneda ka mitte substraadi külge kinnitunult ja vohavad kõrgendatud lämmastiku ja fosfori kontsentratsioonide juures.

Biofiltris kultiveeritud biomassi utiliseerimine on omaette ülesanne, kuna efektiivsel toimimisel võib biomassi juurdekasv olla märkimisväärne (kirjanduse andmetel kuni 10-20 % päevas)(Turan & Neori 2010). Rohevetika biomass võib olla omaette tooraineks paljudele erinevatele ettevõtmistele (Gosh 2012). Esialgselt on ilmselt võimalik kasutada rohevetika biomassi.

Eestis hetkel puudub oskusteave rohevetika toorainena kasutamise kohta, samas on olemas kogemused punavetikate kasutamisel (agariku püüdmine ja geelistuvate ainete tootmine). Mujal maailmas on aga selliseid kogemusi üsna palju ja neid oleks võimalk kindlasti ka Eesti tingimustes kasutada (Lüning & Pang 2003; Titlyanov & Titlyanova 2010).

Eestis on olemas teaduslik taust makrovetikate produktsiooni ja kultiveerimise uurimisel (Kotta et al 2008, Martin et al 2006a, b; Paalme et al 20011, 2013). Senised kogemused ei piirdu agariku ja rohevetikate produktsiooniuuringutega vaid on andmeid ka erinevate teiste taimerühmade produktsiooniuuringute kohta (niitjad rohevetikad, kõrgemad taimed, mändvetikad, pruunvetikad jne.).

Käesoleva projekti tegevuste näol oli tegemist reaalsuslähedase eksperimendi läbiviimisega, kus töötatakse välja ja katsetatakse kalakasvatusest tuleneva kasutatud merevee puhastamist toitainetest kasutades selleks Läänemere makrovetikatel põhinevat biofiltreerimise süsteemi.

Uuring viidi läbi just selle jaoks koostatud eksperimentaalkompleksis, mis seati üles ühe projekti partneri valduses oleval endise kalakasvatuse territooriumil (Kesknõmme kalakasvatus, Saaremaa).

Kavas oli simuleerida väiksemal skaalal reaalse kalakasvatuse projekti järgset tehnoloogiat, kus poolkinnise kalakasvatuse jaoks kasutatakse merevett.

Tegevuse eesmärkideks oli:

- Valida välja sobivad makrovetikaliigid biofiltrites kasutamiseks Läänemere kohalike merevetikate liikide hulgast;
- Kirjeldada nende vetikaliikide inkubeerimiseks vajalikke optimaalseid keskkonnatingimusi;
- Selgitada välja biofiltreerimisel põhineva kalakasvatuse kasutatud merevee toitainete eemaldamise rakendatavus ja tingimused kasutamaks seda tehnoloogiat reaalse kalakasvatuse mastaabi puhul;
- Selgitada välja biofiltreerimise tulemusel kasvatatava vetikabiomassi kasutusvõimalused ja võimalikud piirangud.

Projekt viidi läbi TÜ Eesti mereinstituudi merebioloogia osakonna töörühma poolt koostöös projektipartneritega.

Töödes osalesid:

Georg Martin – projekti juht, proovivõtt, aruandlus
Kristina Tiivel – hangete ettevalmistamine, aruandlus
Martin Teeveer – välitööd, eksperimentaalkompleksi püstitamine
Teemar Püss – eksperimentaalkompleksi püstitamine
Jekaterina Jefimova – keemilised analüüsid
Tuuli Levandi – keemilised analüüsid
Arno Põllumäe – keskkonnaparameetrite mõõtmised
Kaido Noormägi – kompleksi kohapealne käitamine
Jack Hall – välitööd, proovivõtt, vetikabiomassi uuring
Julia Pärn – Tallinna Reaalkooli õpilane, vetikate produktsioonikatsed
Kai Martin – välitööd, laboritööd
Greta Reisalu – välitööd, laboritööd
Hanna Eliisa Luts – välitööd
Kaire Kaljurand – välitööd
Annely Enke – välitööd

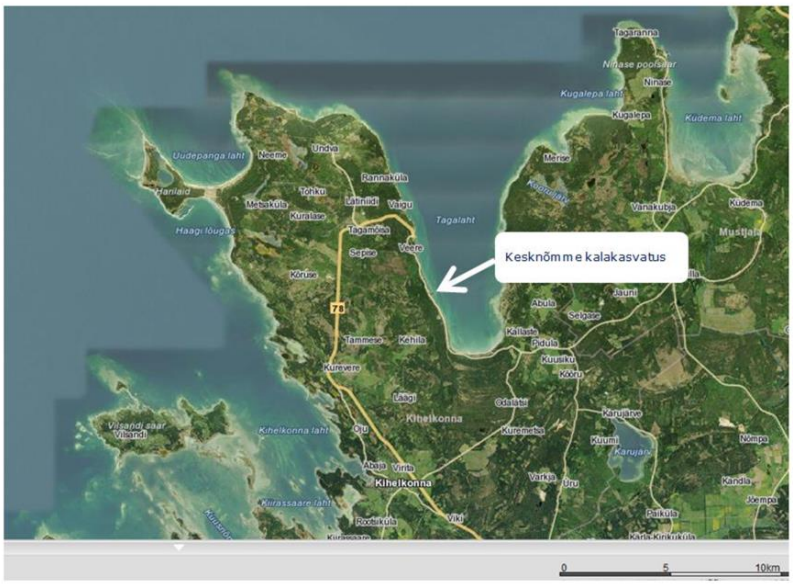
Eksperimentaal-vetikakasvatus Kesknõmme kalakasvatuse territooriumil

Eksperimentaalkompleksi rajamine.

Reaalsusele lähedase katse korraldamiseks oli vaja rajada toimiva kalakasvatuse vähendatud mudel. Selleks koostati eksperimentaalse kompleksi põhimõtteline skeem juba projekti ettevalmistusfaasis. Asukohaks valiti Saaremaa valla Kehila külas Kesknõmmel (katastriüksusel 30101:002:0286) Tagalahe rannal asuv 1980-ndatel rajatud kalakasvatus, mis käesoleval ajal ei toimi tänapäeval sobimatu tehnoloogilise lahenduse tõttu (Joonis 1). Asukoht sobis taolise eksperimendi läbiviimiseks kuna:

- Asukoht oli vahetult mere läheduses (lihtne ligipääs mereveele)
- Sai kasutada endise kalakasvatuse rajatisi ja taristut (elektrivarustus)
- Projekti partneril oli vaba ligipääs territooriumile
- Suletud ja turvatud territoorium.

Eksperiment pidi koosnema mitmest elemendist. Kalakasvatuse komponent – kalamahuti, torustik, veepump merevee pumpamiseks süsteemi, sissetuleva vee aeraator ja filter, kalamahutist väljuva vee jämefilter sette eemaldamiseks. Vetikainkubaatori komponent – mahutid, kus paikneb inkubeeritav vetikabiomass ning torustik vee ärajuhtimiseks. Vesi pumbatakse kalamahutisse (8-9 m^3) pumbaga (maksimaalne jõudlus 26,5 m^3/h). Vetikainkubaatori komponent oli kavandatud koosnema 12-st ristküliku kujulisest mahutist mõõtmetega 16x1,2x06m, mis pidid asetsema jadaühenduses nelja kaupa, et moodustuks neli iseseisvat veevoolu jada. Kolm jada olid mõeldud eksperimentide tarbeks (vetikate inkubeerimiseks) ja üks kontrolliks (vaba veevool).



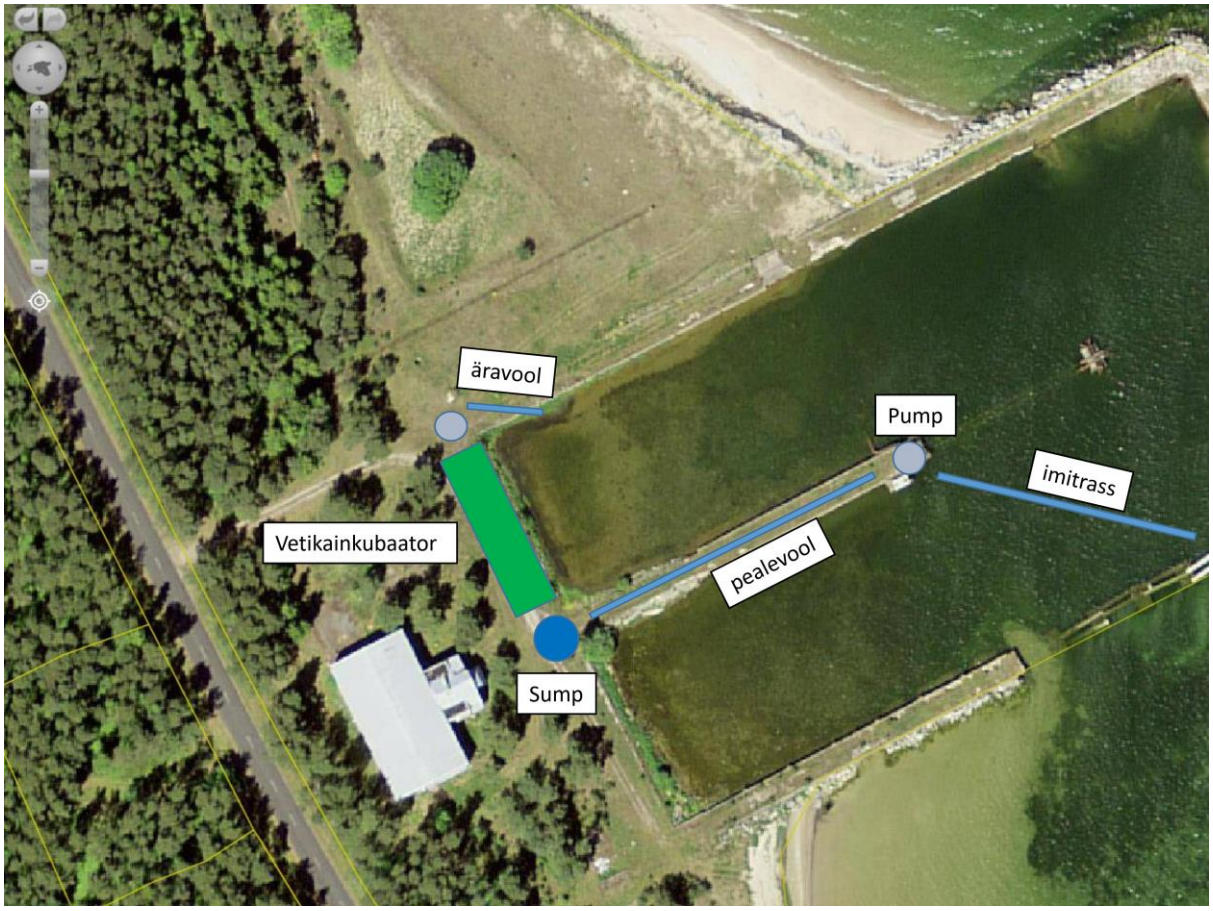
Joonis 1.1 Kesknõmme kalakasvatuse asukoht Tagalahe lääneranniku (aluskaart: Maa-amet 2016)

Eksperimentaalkompleksi esialgne asendiplaan (joonis 2) ja põhimõtteline skeem (joonis 3) tuli hiljem reaalsuses muuta mitmel põhjusel:

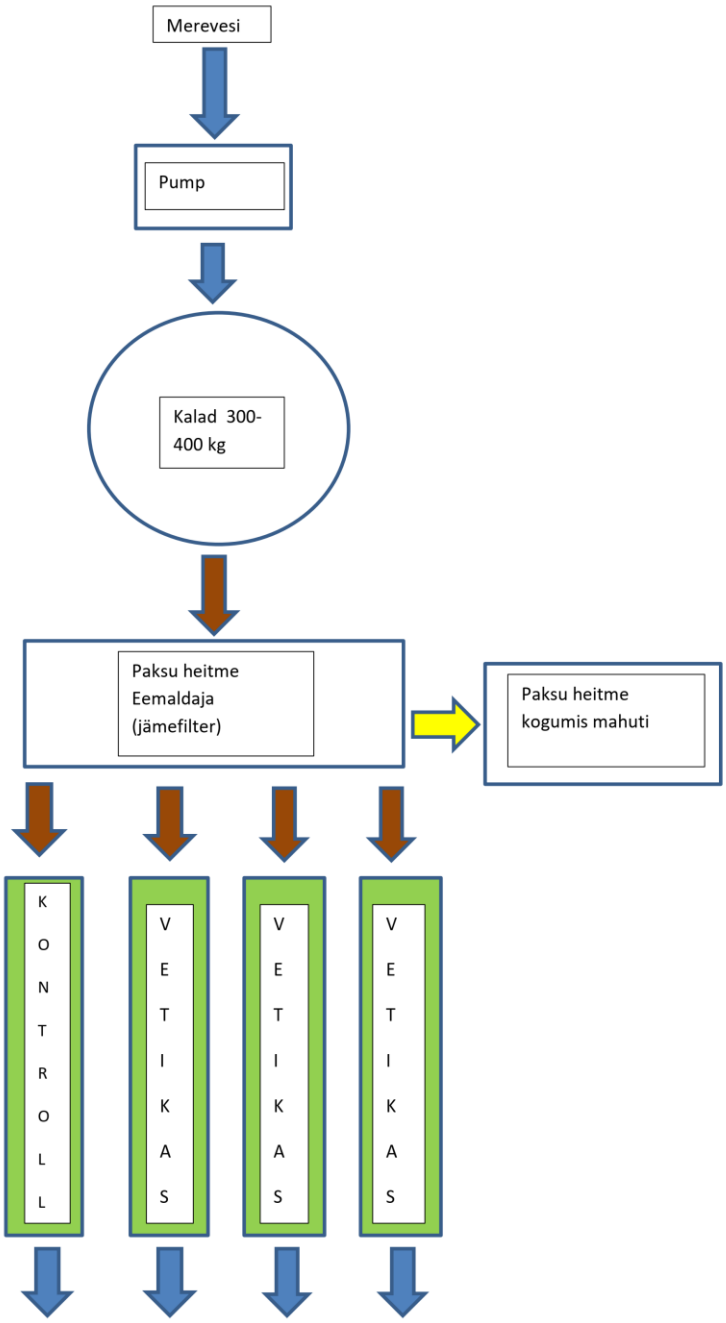
- Esialgne asendiplaan osutus ebamugavaks veevõtu korraldamise osas. Tundus otstarbekam võtta eksperimendi jaoks vesi väljastpoolt basseine (suvisel ajal madalam veetemperatuur).
- Projekti käigus oli võimalus, et territooriumil algavad sinna planeeritud kalakasvatuse ehitustööd ja eksperimentaalkompleks oleks hakanud seda tegevust segama.
- Vetikainkubaatori mahutite hange korduvalt ebaõnnestus, peale mida tuli ümber orienteeruda modulaarsele disainile.

Vetikamahutite paigutus ja konstruktsioon tuli muuta. Esialgu kavandatud 12 mahuti (Joonis 4) asemel võeti kasutusele 64 mahutit 0,8 m^3 ruumalaga, mis olid ühendatud nelja jadasse (Joonis 6).

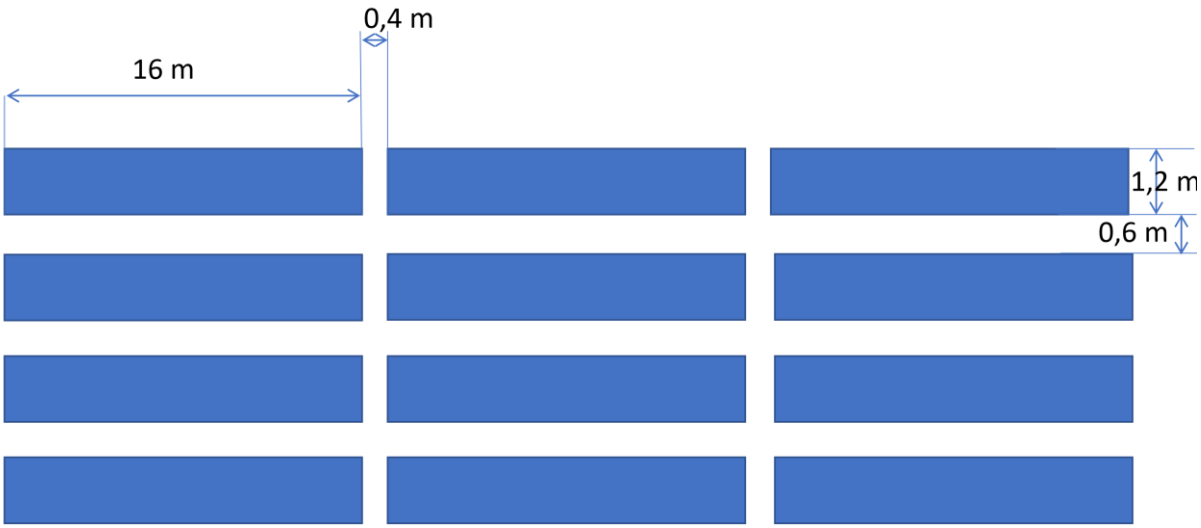
Eksperimendi asukohaskeem muudeti kompaktsemaks (Joonis 5).



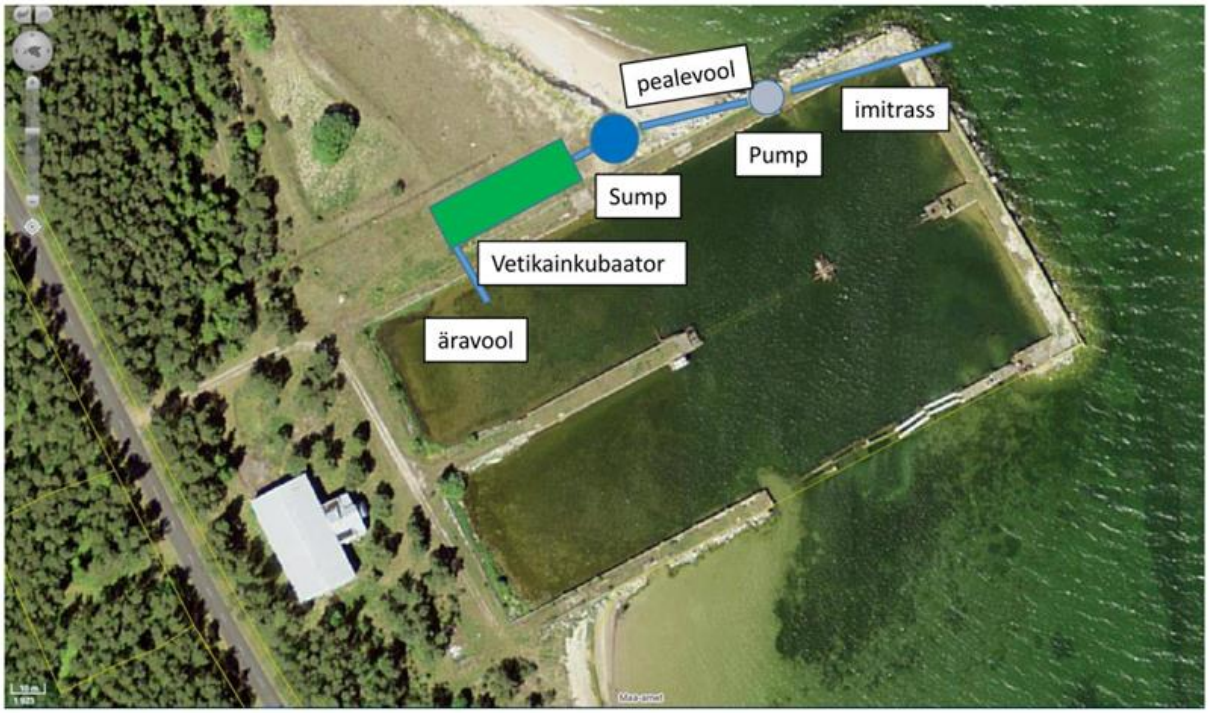
Joonis 2. Eksperimendi paigutusskeem Kesknõmme kalakasvatuse territooriumil – esialgne versioon.



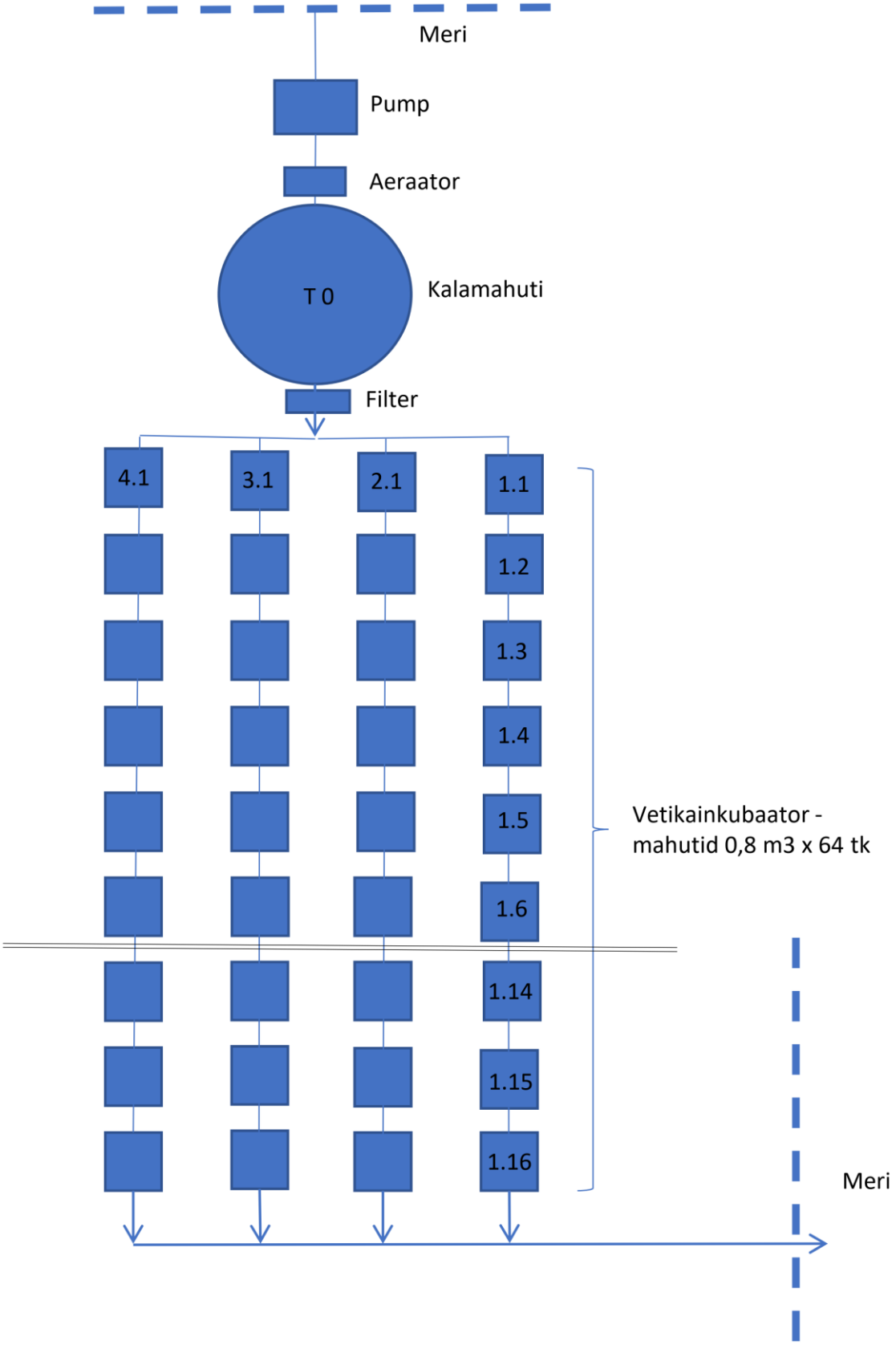
Joonis 3. Eksperimendi põhimõtteline skeem.



Joonis 4. Vetikamahutite paiknemine ja mõõtmed esialgse kava järgi.



Joonis 5. Eksperimendi paigutusskeem Kesknõmme kalakasvatuse territooriumil – realiseerunud versioon.



Joonis 6. Modifitseeritud (realiseeritud) katseskeem koos mahutite markeeringu näidisega.

Eksperimentaalkompleksi rajamine algas 2018. aastal. Kuna 2018. aasta jooksul korduvalt ebaõnnestus vetikainkubaatori mahutite hange, püstitati aasta sügiseks modulaarse süsteemi pilootkatse (Joonis 7-8). See koosnes kalamahutist, filtrist, pumbast ja neljast moodulinkubaatorist. Katse käivitati 2018. aasta oktoobris ilma kalakomponendita. Vetikamahutitesse paigutati söödav rannakarp ja lahtiselt *Ulva intestinalis*. Eksperiment hoiti käigus kaks kuud ja siis konserveeriti.

2019. aasta kevadel laiendati modulaarse vetikainkubaatori osa oluliselt. Lisati ja ühendati kokku 64st moodulist koosnev süsteem (Joonised 9-13). Eksperimentaalkompleks käivitati 24. aprillil. Iga eksperimendijada kahte esimesse mahutisse lisati 3,5 kg söödavat rannakarpi (korjatud Küdema lahest) (Joonis 15). Kalakomponent lisati süsteemi alles sügisel (24.09. asustati mahutisse 230 kg vikerforelli) pärast vee erikasutusloa saamist. Suve esimeses pooles katsetati eri liikide kasvatamist mahutites.

Periood 26.05-03.07 – *Mytilus trossulus* + *Cladophora glomerata*

Periood 22.07-26.08 – *Mytilus trossulus* + *Ulva intestinalis*/ paralleelselt *Ceratophyllum demersum*

Periood 27.08-18.10 – *Mytilus trossulus* + *Ulva intestinalis* + Vikerforell

2020. aasta kevadel täiendati süsteemi õhutusega. Kompressoriga ühendati igast eksperimendijadast kolm mahutit (vastavalt iga jada nr 3, 4, 5). Õhutus toimis intervalliga igas tunnis 20 min. Algusest peale kasutati ka kalakomponenti (suve jooksul hoiti kalamahutis 30-50 kg vikerforelli).

2020. aasta jooksul teostati kokku kolm biomassi juurdekasvu eksperimenti:

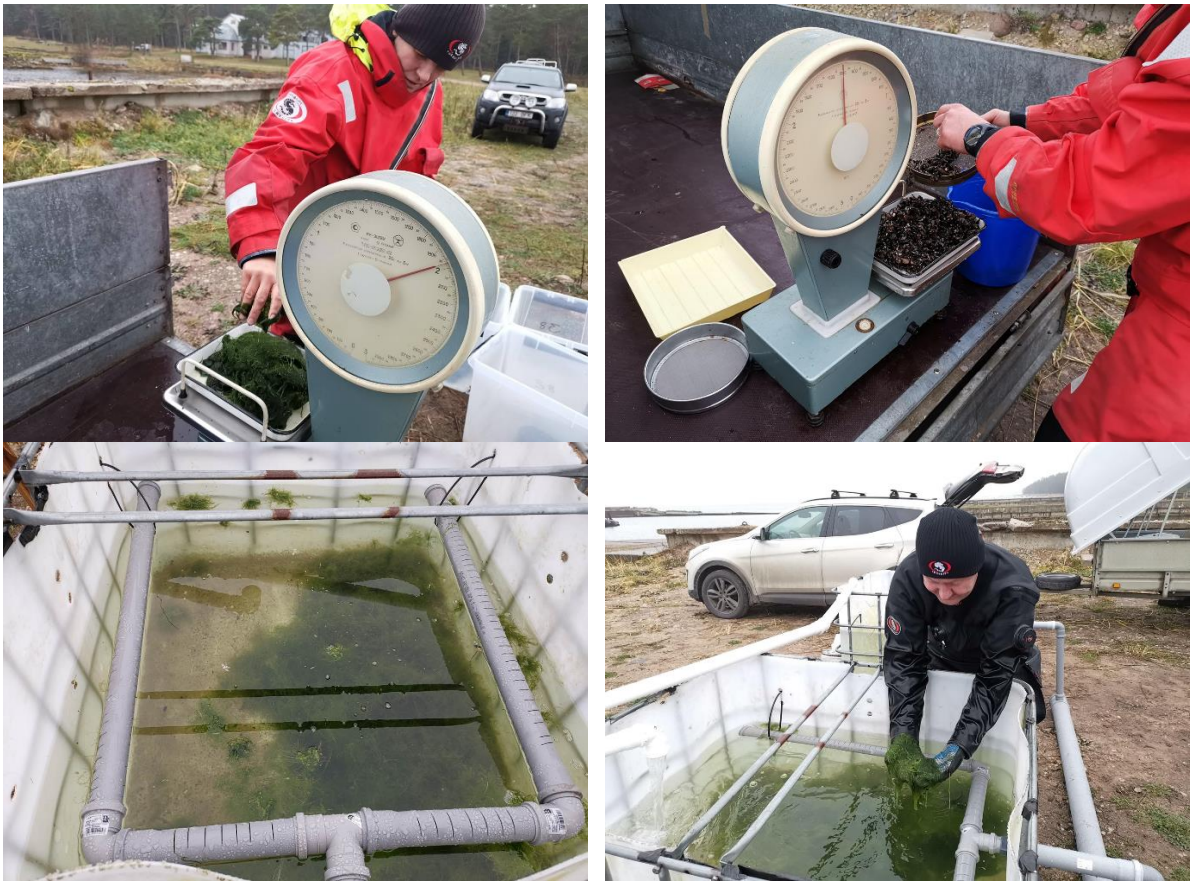
26.05-25.06 – 30 päeva

21.07-12.08 – 22 päeva

04.09-01.10 – 27 päeva

Need katsed olid suunatud toitainete eemaldamise ja biomassi juurdekasvu hindamisele. Inkubeeriti erinevaid koguseid *Ulva intestinalist* ja kalamahutis hoiti stabiilselt 30-50 kg vikerforelli.

Joonis 7. Pilootkatse seadistamine (oktoober 2018).



Joonis 8. Pilootkatse ülespanek (oktoober 2018).

Joonis 9. Eksperimendi seadistamine (aprill 2019). Vaade meresuunast.

Joonis 10. Eksperimendi seadistamine (aprill 2019).

Joonis 11. Eksperiment külgvaates (september 2020).

Joonis 12. Eksperimendi seadistamine (aprill 2019). Paremal kalamahuti ja jämefilter.

Joonis 13. Eksperimendi seadistamine (aprill 2019). Sissetuleva vee aeraator ja sademefilter.

Joonis 14. Mahutite markeeringu näidis.

Joonis 15. Söödava rannakarbi istutamine süsteemi (aprill 2019).

Keskkonnaparameetrite mõõtmine

Kogu eksperimendi käigus mõõdeti järgmisi veekeskkonna füüsikalisi ja keemilisi parameetreid:

- Vee temperatuur (pidevmõõtmised)
- Vee hapnikusisaldus (pidevmõõtmised automaatlogeriga, kord kuus käsianduriga)
- pH (automaatmõõtmised logeriga)
- Soolsus (iganädalaselt)
- Nitraadi kontsentratsioon (regulaarselt eksperimentide ajal)
- Nitriti kontsentratsioon (regulaarselt eksperimentide ajal)
- Fosfaadi kontsentratsioon (regulaarselt eksperimentide ajal)
- Üldfosfori kontsentratsioon (regulaarselt eksperimentide ajal)
- Üldlämmastiku kontsentratsioon (regulaarselt eksperimentide ajal)

Lisaks mõõdeti vee erikasutusloa seiretingimustele vastavalt järgmisi parameetreid:

- KHT (kord kuus kalakasvatuse perioodil)
- BHT (kord kuus kalakasvatuse perioodil)
- Heljum (kord kuus kalakasvatuse perioodil)

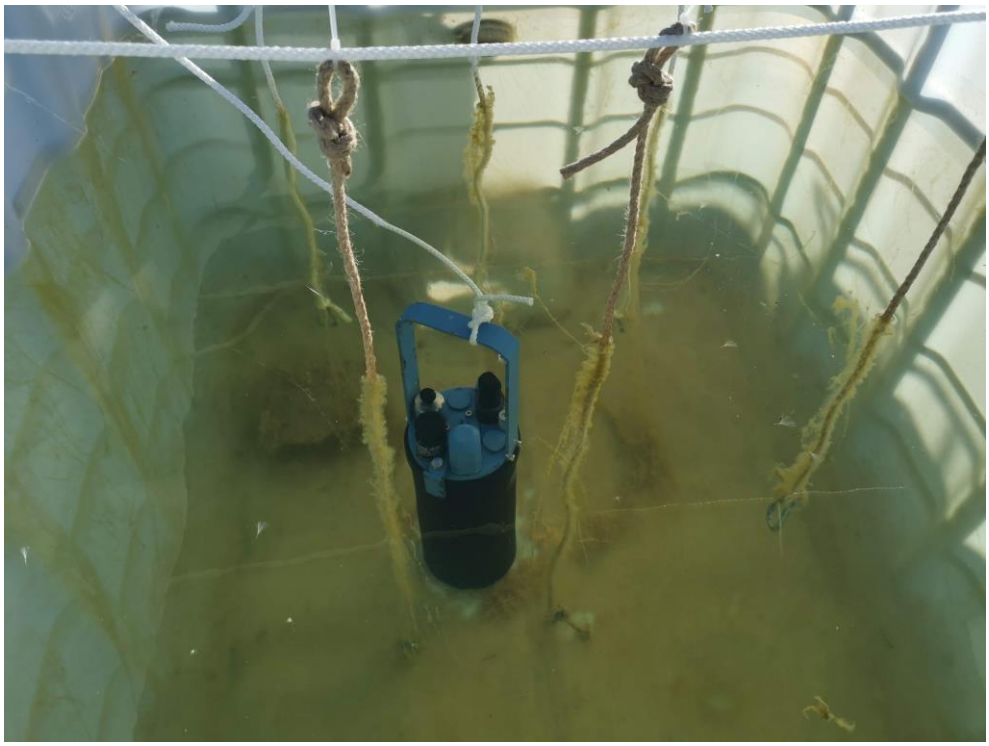
Proovid keemiliste analüüside teostamiseks koguti järgmistelt:

2018	**2019**	**2020**	**Vee erikasutusluba**
Sissevool	Kalamahuti (T 0)	Kalamahuti (T 0)	Sissevool (INLET)
Kalamahuti (T 0)	1.2; 2.2; 3.2; 4.2	1.3; 2.3; 3.3; 4.3	Pärast jämefiltrit (AFT)
Mahuti 1	1.4; 2.4; 3.4; 4.4	1.5; 2.5; 3.5; 4.5	Väljavool (OUTLET)
Mahuti 2	1.8; 2.8; 3.8; 4.8	1.9, 2.9; 3.9; 4.9	
Mahuti 3	1.12; 2.12; 3.12; 4.12	1.15; 2.15; 3.15; 4.15	
Mahuti 4	1.16; 2.16, 3.16; 4.16		

Lisaks teostati perioodiliselt ka CO_2 mõõtmisi nii looduslikes kooslustes kui inkubatsioonimahutites.

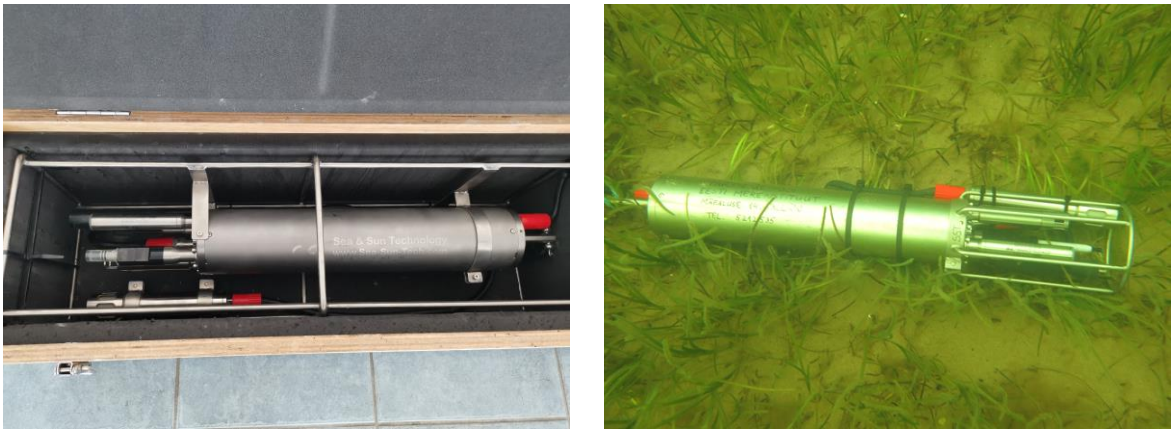
Keemilised analüüsid teostati Tartu Ülikooli Eesti Mereinstituudi Merebioloogia osakonna laboris, mis on EAK poolt akrediteeritud katselabor registreerimisnumbriga L179 (13.12.2025)

Joonis 16. Hapnikulogerite kalibreerimine.



Joonis 17. Vee parameetrite mõõtmine CTD sondiga 2019. aasta mais.

Joonis 18. Automaatsete valgus- ja temperatuurilogerite paigaldamine mahutitesse (2020. aasta esimene eksperiment, 08.06.2020).



Joonis 19. SSN M90 sond, mida kasutati loodusliku vetikakoosluse keskkonnatingimuste kirjeldamiseks ja inkubeerimismahutite eri parameetrite jälgimiseks.

Joonis 20. Eksperimendi käigu seireks kasutatud autonoomsed hapnikuloger (vasakul) ja pH loger (paremal).

Kultiveerimiseks sobiva vetikaliigi valik

Kultiveerimiseks sobiva vetikaliigi valikul lähtuti mitmest kriteeriumist:

- Liik peab olema kohalik ja kohastunud kasvama riimvees;
- Liik võiks olla kohastunud taluma või soosima kõrgemaid toitainete kontsentratsioone;
- Liik peab olema võimeline kasvama ka kinnitumata substraadile;
- Liik peab olema suhteliselt kiire kasvuga;
- Liiki peaks olema võimalik suhteliselt lihtsalt merest koguda;
- Liik peab olema vastupidav fragmenteerumisele;
- Liik võiks olla tööndusliku kasutatav mujal maailmas.

Lähtudes ülaltoodud kriteeriumitest töötati läbi teaduslik kirjandus Läänemeres levivatest makrovetikaliikidest ja koostati nimekiri, mida siis analüüsiti edasi praktilise rakendatavuse osas.

Liigid, mida kaaluti katsesse kaasamiseks, aga mis teatud põhjustel katsesse ei valitud:

Põisadru (*Fucus vesiculosus*) – on laialt levinud liik, kohastunud elama madalas rannikumeres, esineb ka lahtisel kujul, tal on tööndusliku kasutamise potentsiaal. Katsesse ei sobinud kuna – aeglane kasv (kirjanduse andmetes ja varasemate produktsioonimõõtmiste kohaselt on juurdekasv maksimaalselt 10-15% biomassi aastas), liik on pigem madalate toitainete kontsentratsioonide eelistaja.

Agarik (*Furcellaria lumbricalis*) – samuti laialt levinud liik, võib esineda lahtiselt substraadile kinnitamata, paljuneb vegetatiivselt ja talub fragmenteerumist, omab tööndusliku väärtust ja kasutust ka Eestis. Katsesse ei sobinud kuna – aeglane juurdekasv (kuni 100% aastas ideaaltingimustes), liik on kohastunud elama sügavamal ja seega valgustingimused avamaal mahutites kultiveerimisel vajavad reguleerimist, ei talu kõrgeid veetemperatuure.

Ceramum tenuicorne – laialt levinud niitja tallusega liik, esineb erinevatel sügavustel ja võib kasvada ka lahtiselt substraadile kinnitumata. Katsesse ei sobinud eelkõige selle tõttu, et seda liiki on väga raske koguda katse jaoks vajalikus koguses.

Pilayella littoralis – laialt levinud niitja tallusega pruunvetika liik, esineb erinevatel sügavustel ja võib kasvada ka lahtiselt substraadile kinnitumata. Katsesse ei sobinud selle tõttu, et seda liiki on raske koguda katse jaoks piisavas koguses.

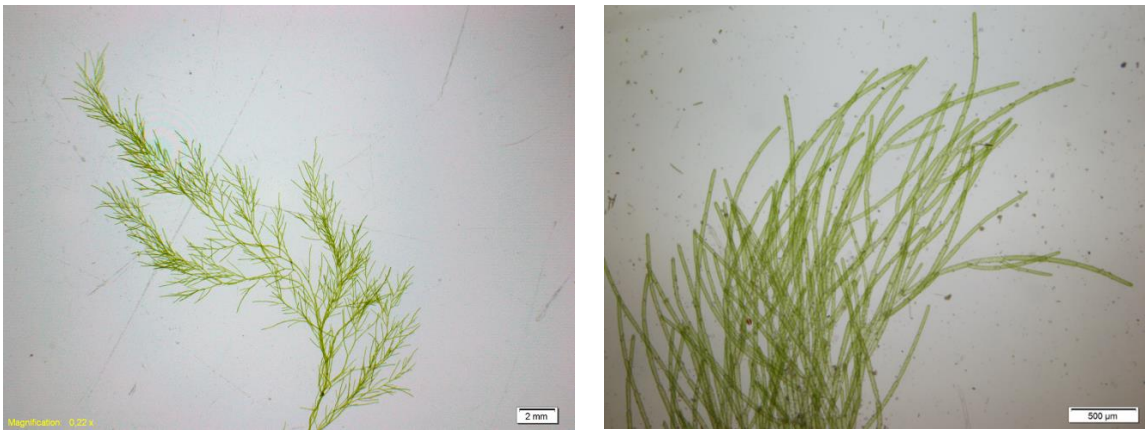
Merihein (*Zostera marina*) - laialt levinud Eesti rannikumeres, omab potentsiaali tööndusliku kasutuseks, on olemas kogemus ümberistutamiseks teiste varasemate projektide käigus. Katsesse jäi valimata eelkõige katse tehnilise lahenduse tõttu. Liik vajab kasvamiseks merepõhja kinnitumist ja kasutatud katse teostus sellist lahendust ei võimaldanud.

Liigid, mis valiti katsesse:

Cladophora glomerata, *Ulva intestinalis* ja *Ceratophyllum demersum*.

Cladophora glomerata.

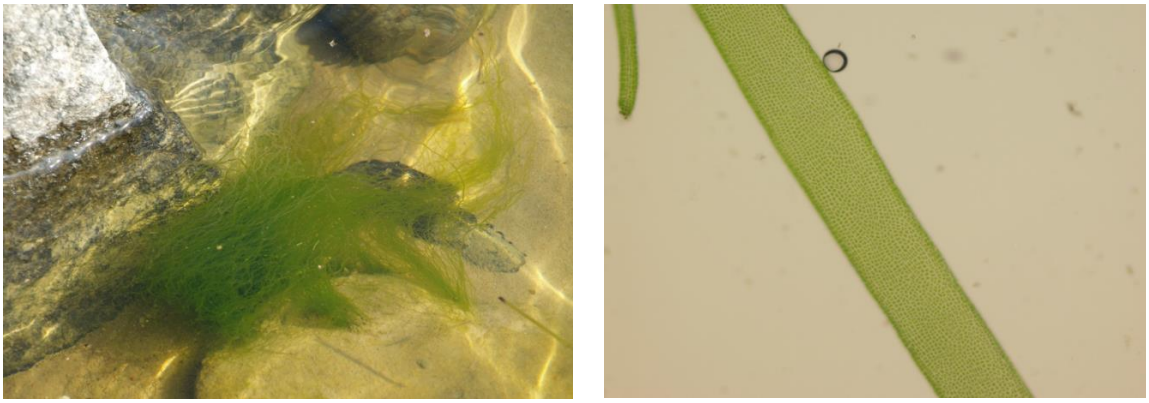
Tegemist on väga laialt levinud rohevetikaliigiga (Joonis 21). Mageveeliik, mis kasvab ka mõõdukalt soolases riimvees. Eesti rannikumeres on see liik äärmiselt laialt levinud. Liik on sesoonne – massiliselt esineb suve alguses. Hiljem esineb kogu vegetatsiooniperioodi jooksul ja võib põhjustada ka mitu õitsengut (massesinemist) suve jooksul. Liik on väga kiire kasvuga ja kõrge primaarproduktsiooni aktiivsusega. Kohastunud taluma lauspäikest ja kõrgemaid veetemperatuure madalas vees. Looduslikult kasvab madalas vees, kinnitub nii vabale substraadile kui ka teistele taimedele. Substraadi küljest lahti rebitult võib moodustada vetikamatte. Tööndusliku kasutamise osas võib pakkuda huvi nanotselluloosi toorainena.



Joonis 21. Rohevetikaliik *Cladophora glomerata* mikroskoobis.

Ulva intestinalis

Tegemist on väga lihtsa ehitusega rohevetikaliigiga, mis asustab kõva substraati vahetult veepiiril (Joonis 22). Liik on algselt pärit merelisest keskkonnast, kuid saab väga hästi hakkama ka madala soolsuse juures. Tänapäeval asustab see liik kõiki meresid ja on leitav kõikides ookeanides. Liiki esineb praktiliselt aasta läbi, rohkem esineb teda kevadisel ja hilissuvisel perioodil. Taim kasvab n-ö. lõpmatult kuni tema tallus laguneb lainetuse mõjul. Võib kasvada ka vabalt hõljuvalt, ei vaja kinnitumist substraadile. Liik on väga kiire kasvuga, eelistab toitaineterikast vett. Liiki kasutatakse inimtoiduks väga laialdaselt mujal maailmas. Oma kiire kasvu tõttu kasutatakse seda liiki ka mujal vesiviljeluses ja samuti on näiteid, kus seda liiki kasutatakse reovee puhastamisel.



Joonis 22. Rohevetikaliik *Ulva intestinalis*.

Ceratophyllum demersum

Räni-kardhein (*Ceratophyllum demersum*) on veesisene taim kardheinaliste sugukonnast kardheina perekonnast (Joonis 23). Räni-kardhein on kosmopoliitse levikuga mageveetaim.

Enamasti kasvab ta toiteaineterikastes seisvates või väga vaikse vooluga veekogudes suvise temperatuuriga 15–30 °C, kinnitudes mudasele põhjale. Vahel esineb ta ka nõrgalt riimveelistes veekogudes. Taime vars kasvab 1–3 m pikkuseks. Varrel on hulgaliselt külgvõsusid, mis muudab ühe isendi suureks põõsakujuliseks massiks. Iga leht on 8–40 mm pikk. Lehed on 6–12 kaupa männastes. Lehed on terava servaga hammastega, jäigad ja haprad.

Taim on ühekojaline ja õitseb juulist septembrini. Õied on väikesed, umbes 2 mm pikad, kroonlehti on 8 või rohkem ja need on rohekaspruunikad. Samal taimel moodustuvad eraldi isas- ja emasõied.

Räni-kardheina kasvatatakse sageli akvaariumides, sealhulgas külma veega täidetud akvaariumides. Isegi ilma juurteta võib taim kinnituda akvaariumi põhja või akvaariumis olevate esemete külge.

Räni-kardheina kasutatakse veekogude puhastamiseks, sest nad eritavad aineid, mis takistavad tsüanobakterite arengut.

Joonis 23. Räni-kardhein *Ceratophyllum demersum*.

Katsed inkubeerimiseks sobiva liigi valikuks

Katsed sobiva liigi valikuks kalakasvatuse vee puhastamiseks vetikainkubaatoris viidi läbi 2019. aasta suve perioodil. Katsed olid korraldatud selliselt, et kogu süsteem hoiti töös teatud perioodil jälgides nii taimede seisundit kui vee parameetrid. Perioodiliselt hinnati taimede seisundit – selleks teostati nii visuaalsed vaatlused kui ka mõõdeti taimede füsioloogilist seisundit primaarproduktsiooni aktiivsuse hindamise kaudu.

Eksperimentide eesmärgiks oli võimalikult pika perioodi jooksul hoida inkubeeritav vetikabiomass elus ja leida vetikabiomassi kultiveerimiseks sobivaimad keskkonnatingimused.

Kõikide eksperimentide ajal hoiti süsteemis ka mahuteid söödava rannakarbiga. Seda tehti eeldades, et söödava rannakarbiga asustatud mahutid toimivad kui esmane toitainete ja lahtise sette püüdur. Söödav rannakarp on aktiivne filtreerija, ta püüab vees oleva heljumi ja tarbib selle oma toiduks, töötades nii orgaanika ringi taimedele paremini omastatavasse vormi.

Katseperiood 26.05-03.07.2019 – *Mytilus trossulus* + *Cladophora glomerata*

Esimese katseperioodiga alustati maikuu lõpus kui lisati katsesse Tagalahest kogutud *Cladophora glomerata*. Söödav rannakarp oli süsteemis juba selleks ajaks ligi kuu aega (karbid lisati esimestesse mahutitesse 24.04.2019). Süsteem töötas tühjalt, veevoolu kiirus oli 1/10 maksimaalvõimsusest.

Eksperiment oli üles ehitatud selliselt, et kalamahutist tulev vesi suubus alguses esimese rea mahutitesse. Söödav rannakarp asustati teise ja kolmanda rea mahutitesse igasse 3,5 kg (vastavalt mahutid 1.2 ja 1.3; 2.2 ja 2.3; 3.2 ja 3.3). Vetikas *Cladophora glomerata* asustati viienda ja kuuenda rea mahutitesse igasse 6 kg märgkaalus (1.5 ja 1.6; 2.5 ja 2.6, 3.5 ja 3.6).

Eksperimendi käik ja tulemuste kokkuvõte. Eksperimendi esimestel nädalatel püsis vee temperatuur alla 15 kraadi. Alates 04.06 on aga ilmastikutingimused muutunud ja vee temperatuur hakkab mahutites kiirelt kasvama. Samuti oli probleemiks esimesel nädalal vetikamassi hoidmine mahutis. Vetikamass hakkas koos veevooluga liikuma mööda mahutite jada ja kaod olid märkimisväärsed.

Cladophora hakkas lagunema juba alates teisest nädalast (kõrval olev foto mahutist 1.5 on tehtud 05.06). Mahutites kerkis surnud vetikamass pinnale ja hakkas roiskuma. Kuni juuni keskpaigani oli mahutites pindmise surnud vetikamassi all ka elusat materjali. Eksperimendi lõpuks oli enamus Cladophora massist surnud.

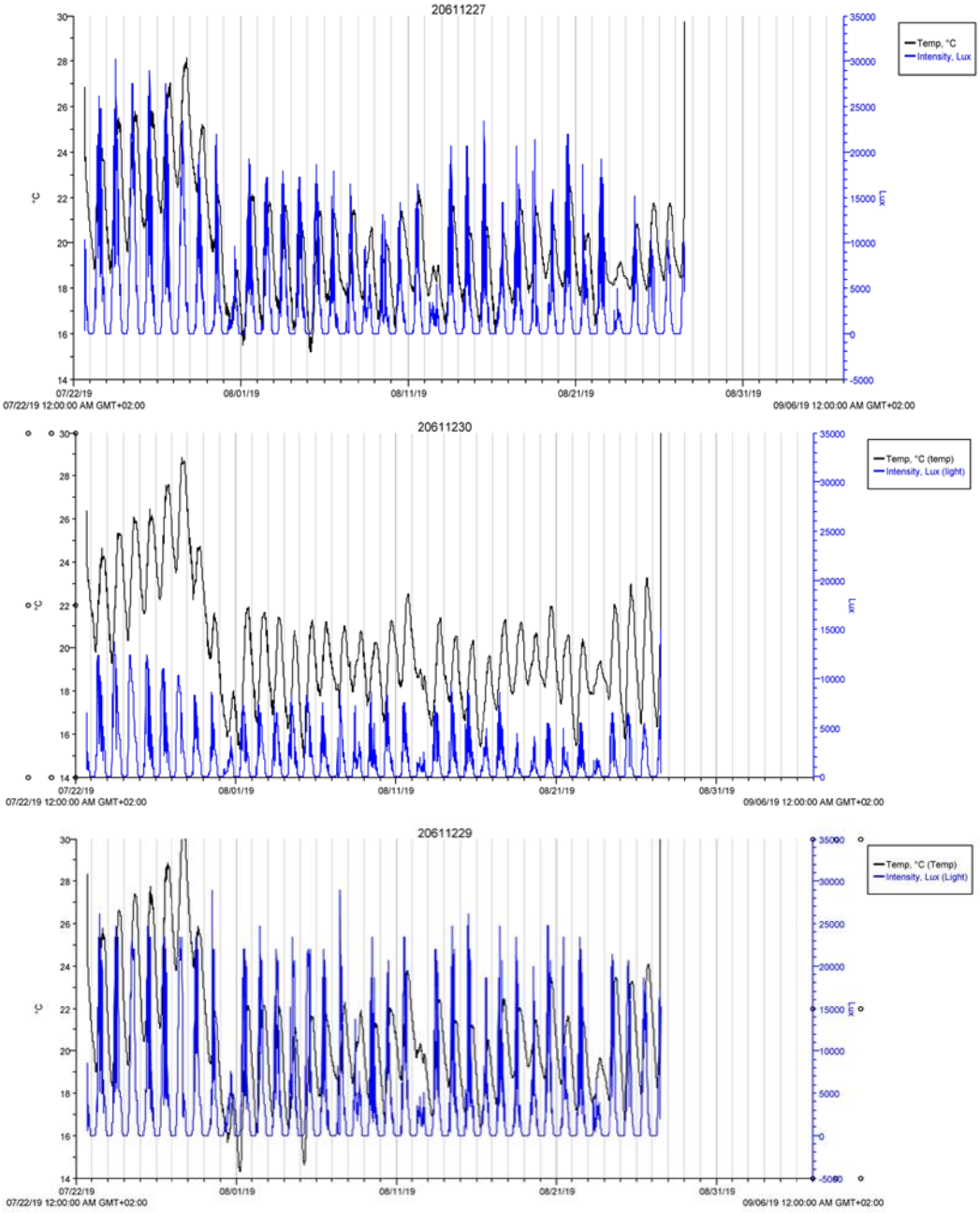
Ka toitainete kontsentratsiooni analüüsid näitasid, et kohe peale vetikate ja rannakarpide asustamist süsteemi hakkas biomassist lekkima toitaineid (LISA 2 joonised 1-4). Eksperimendis kasutatud söödav rannakarp pidas eksperimendi aja väga hästi vastu ja loomad vabastati pärast eksperimenti.

Katseperiood 22.07-26.08.2019 – *Mytilus trossulus* + *Ulva intestinalis*/paralleelselt *Ceratophyllum demersum*

Teise katseperioodi eesmärk oli katsetada teiste liikidega. Seekord koguti Küdema lahest Saaremaa sadama juurest piisav kogus *Ulva intestinalst* ja söödavat rannakarpi. Söödav rannakarp paigutati teise, kolmanda ja neljanda rea mahutitesse (vastavalt mahutid 1.2, 1.3 ja 1.4; 2.2, 2.3 ja 2.4; 3.2, 3.3 ja 3.4). Igasse mahutisse paigutati umbes 2 kg söödavat rannakarpi. Ulva paigutati viienda rea mahutitesse (1.5; 2.5; 3.5). Mahutitejadade viimastes mahutites toimus ka paralleeleksperiment Räni kardheinaga.

Eksperimendi käik ja tulemuste kokkuvõte. Ulva pidas vastu oluliselt paremini kui eelmises eksperimendis olnud Cladophora. Oli samuti probleeme vetikamassi hoidmisega inkubatsioonimahutis. Kuigi vee temperatuurid kerkisid juuli lõpuks väga kõrgele (Joonis 23) jäi Ulva ellu. Süsteem toimis ka toitainete ärastajana – mitmed keemia parameetrid näitasid, et süsteemist läbivoolavast veest eemaldatakse toitaineid (LISA 2 joonised 5-6).

Paralleelselt läbiviidud katse Räni kardheinaga ebaõnnestus, kuna katsematerjal hävis esimese kahe nädala jooksul. Tõenäoliselt ei pidanud taimed vastu kõrgele temperatuurile.



Joonis 23. Valguse ja temperatuuri logeri salvestused teise eksperimendi ajal (loger 20611227 – mahuti 1.1; loger 20611230 – mahuti 1.4; loger 20611229 – mahuti 1.15).

Katseperiood 27.08-18.10.2019 – *Mytilus trossulus* + *Ulva intestinalis* + Vikerforell

Kolmanda katseperioodi eesmärgiks oli kontrollida valitud liigi sobivust inkubeerimiseks lisades eksperimenti ka kalakomponendi. Erinevuseks eelmise perioodiga on ka veevoolu maht, mis suurendati täisvõimsuse peale. Söödav rannakarp paigutati teise, kolmanda ja neljanda rea mahutitesse (vastavalt mahutid 1.2, 1.3 ja 1.4; 2.2, 2.3 ja 2.4; 3.2, 3.3 ja 3.4). Igasse mahutisse paigutati umbes 2 kg söödavat rannakarpi. Ulva paigutati viienda ja kuuenda rea mahutitesse (1.5 ja 1.6; 2.5 ja 2.6; 3.5 ja 3.6). Ulva kogus oli umbes 3 kg märgkaalus igasse mahutisse.

Eksperimendi käik ja tulemuste kokkuvõte. Vee temperatuur langes eksperimendi jooksul oluliselt. Kui eksperimendi algusperioodil oli veetemperatuur +20 kraadi juures, siis eksperimendi lõpus oli vee temperatuur langenud +11 kraadi juurde. Eksperimendi jooksul oli Ulva olukord stabiilne, vetikabiomassi lagunemise märke polnud.

Katsetatud vetikaliikide primaarprodukstsiooni mõõtmised

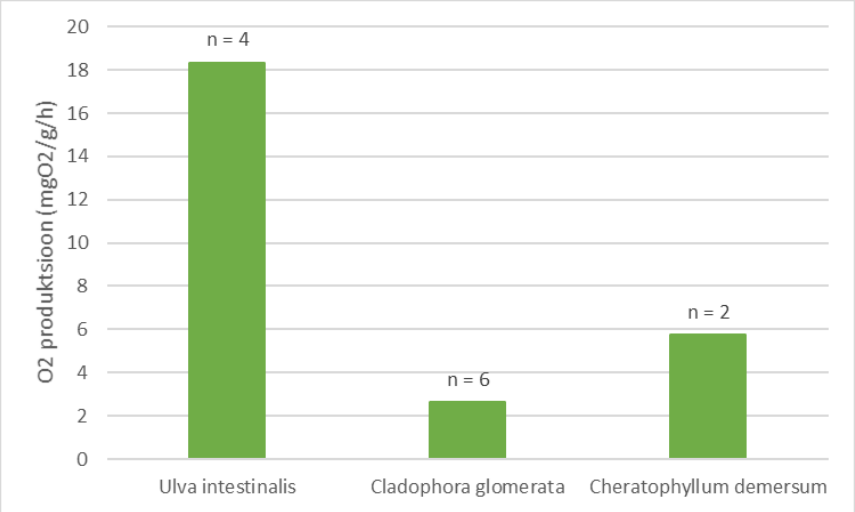
Kõiki kolme katsetatud taimeliiki hinnati ka fotosüsteetilise aktiivsuse seisukohalt. Primaarproduktsiooni mõõdeti hapnikumeetodil inkubeerides katsematerjali kindla aja jooksul katsepudelis ja registreerides hapnikukontsentratsiooni katse alguses ja lõpus (joonis 24). Selle abil saab hinnata taime füsioloogilist seisundit – kui fotosüntees on aktiivne on taimel keskkonnatingimused optimaalsed. Samuti näitab see põhimõtteliselt ka taime fotosünteesi aktiivsust ja aitab ka kaudselt hinnata biomassi juurdekasvu potentsiaali.

Tehtud mõõtmised näitasid, et kõige suurema aktiivsusega fotosünteesis *Ulva intestinalis* (joonis 25).

Tehtud katsete põhjal valiti järgmisteks katseteks sobivaks liigiks *Ulva intestinalis*:

- See liik oli tunduvalt vastupidavam avamaal lahtistes mahutites inkubeerimiseks
- See liik näitas tunduvalt suuremat fotosünteetilist aktiivsust ja biomassi juurdekasvu

Joonis 24. Inkubeeritavate liikide primaarproduktsiooni mõõtmine.



Joonis 25. Inkubeeritavate liikide füsioloogilise seisundi kontrolliks hinnatud primaarproduktsiooni aktiivsus nädal pärast inkubatsiooni algust. Mõõtmised on teostatud erineval ajal.

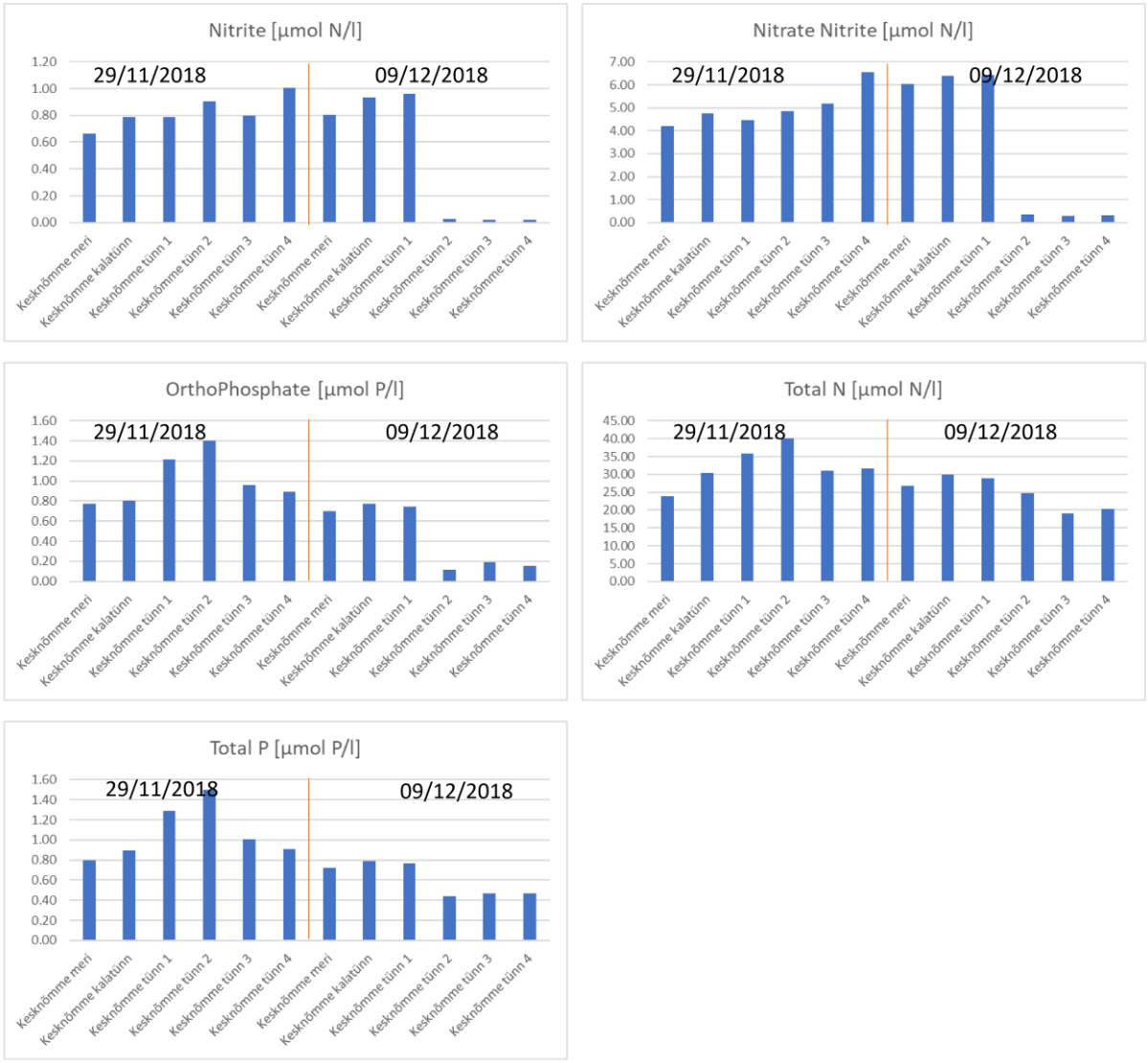
Kogu projekti põhieesmärgiks on demonstreerida merevee läbivoolul põhineva kalakasvatuse heitvee puhastamist/liigsete toitainete eemaldamist kasutades selleks makrovetikatel põhinevat biofiltersüsteemi.

Selleks teostati rida eksperimente, kus inkubeeriti merest kogutud vetikamaterjali selleks koostatud eksperimentaalkompleksis.

Kogu süsteemi kontseptsiooni katsetamiseks viidi läbi esimene pilootkatse 2018. aasta hilissügisel. Eksperimentaalkompleks koosnes siis kalamahutist, pumbast, jämefiltrist ja neljast inkubatsioonimahutist (Joonis 7). Inkubatsioonimahutisse paigutati 09.11.2018 igasse kuni 2 kg söödavat rannakarpi ja 3 kg *Ulva intestinalist* (Joonis 25). Eksperiment toimus hilissügisel, vee temperatuur oli 10-15 kraadi vahel.

Analüüsid toitainete kontsentratsioonide määramiseks võeti kahel korral (29.11 ja 09.12). Esimesel korral näitasid analüüsid kõrgemaid toitainete kontsentratsioone inkubatsioonimahutites võrreldes süsteemi sissetuleva veega. Samas teine analüüside seeria näitas inkubatsioonimahutites oluliselt madalamaid kontsentratsioone kui süsteemi sissetulevas vees (10-15 % üldlämmastiku puhul ja 30-40 % üldfosfori puhul; joonis 26; andmed LISA 1 Tabel 1). Esimese analüüside seeria ajal võis olla tegemist katse ülespanekuga katsesse toodud kahjustada saanud nii rannakarbi kui Ulva lagunemisest tuleneva täiendava toitainete vooga. Teine analüüside seeria näitas, et selline süsteem võib toimida läbivoolavast veest toitainete eemaldajana.

Joonis 25. *Ulva intestinalis* inkubatsioonimahutis 09.11.2018.



Joonis 26. 2018 aastal teostatud pilootkatse veekeemia analüüside tulemused.

Selgitamaks vetikatel põhineva biofiltersüsteemi efektiivsust viidi 2020. aastal läbi kolm eksperimentide seeriat:

26.05-25.06 – kestvus 30 päeva

21.07-12.08 – kestvus 22 päeva

04.09-01.10 – kestvus 27 päeva

Eksperimentide eesmärgiks oli jälgida kalakasvatusmahutist pärineva vee toitainete kontsentratsiooni muutust biofiltersüsteemis. Selleks koguti veekeemia proove kõigist neljast mahutite jadast ning kalakasvatusmahutist. Lisaks teostati ka kord kuus vee erikasutusloa järgset seiret, kus määrati lisaparameetreid sissetulevast veest, vetikainkubaatorisse sisenevast veest ja süsteemist väljuvast veest. Eksperimendi käigus jälgiti ka mahutites oleva vee temperatuuri ja soolsuse muutumist. Samuti registreeriti automaatlogeritega ka pH ja hapnikusisaldust nii kontrolljadas kui vetikatega asustatud mahutites. Eksperimendi alguses ja lõpus määrati ka mahutites oleva vetikamassi märgkaal ja seeläbi oli võimalik hinnata vetikamassi juurdekasvu. Kalade kogus oli kõikide eksperimentide käigus sama

(asustati 30 kg). Kalade suremus oli väike, kogu eksperimendi käigus oli kalade kadu suremusena kuni 5 %.

Veevool hoiti kõikide eksperimentide käigus maksimaalne, samas esines ka rikkeid ja voolukatkestusi.

Mahutid vetikatega olid aereeritud suruõhukompressoriga. Aereerimine toimus perioodiliselt 20 min jooksul igas tunnis.

Töö käik ja tulemused.

Veetemperatuur mahutite jadades muutus sõltuvalt ilmastikutingimustest, veetemperatuuri erinevus esimeste ja viimaste mahutite vahel võis olla kuni 3 kraadi (Joonised 27-29). Kõrgeimad veetemperatuurid ulatusid juunikuus 25 kraadini. Valgus- ja temperatuurilogeri salvestuses registreeriti maksimaalne veetemperatuur juuni lõpus isegi 26 kraadi (Joonis 30). Samas merevee soolsus püsis kogu eksperimendi perioodi jooksul väga stabiilsena.

Kõrge veetemperatuur pärsib vetikate füsioloogiat ja võib tekitada hapniku puuduse vetikamassis. Meie eksperimendi puhul olu näha kõrge temperatuuri pärssivat mõju Ulva biomassile, esimese eksperimendi lõpuks (26.06) oli enamus mahutites hoitud vetikamassist surnud. Kõrge temperatuuri pärssiv mõju biofiltersüsteemi toimimisele avaldus ka keemia analüüside tulemustes.

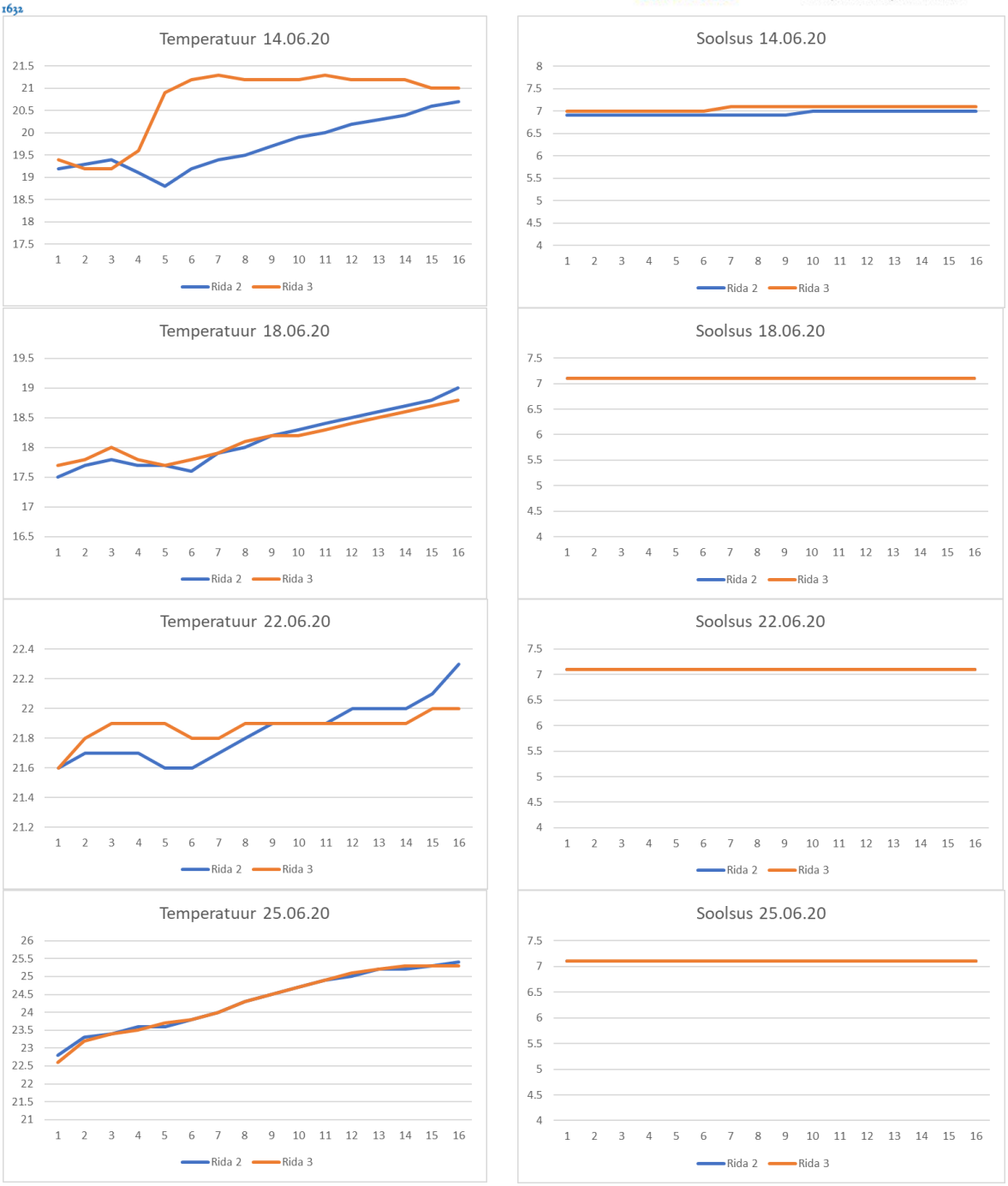
Probleeme tekkis ka teise eksperimendi käigus, kus vahetult enne eksperimendi lõppu seiskus pumba rikke tõttu veevool mitmeks päevaks. See avaldas lõppkokkuvõttes pärssivat mõju vetikabiomassi juurdekasvule.

Kõige õnnestunumaks eksperimendiks osutus kolmas eksperiment. Selle käitamise käigus olid nii veetemperatuurid mõõdukad, tehniliselt toimis süsteem ilma suuremate tõrgeteta.

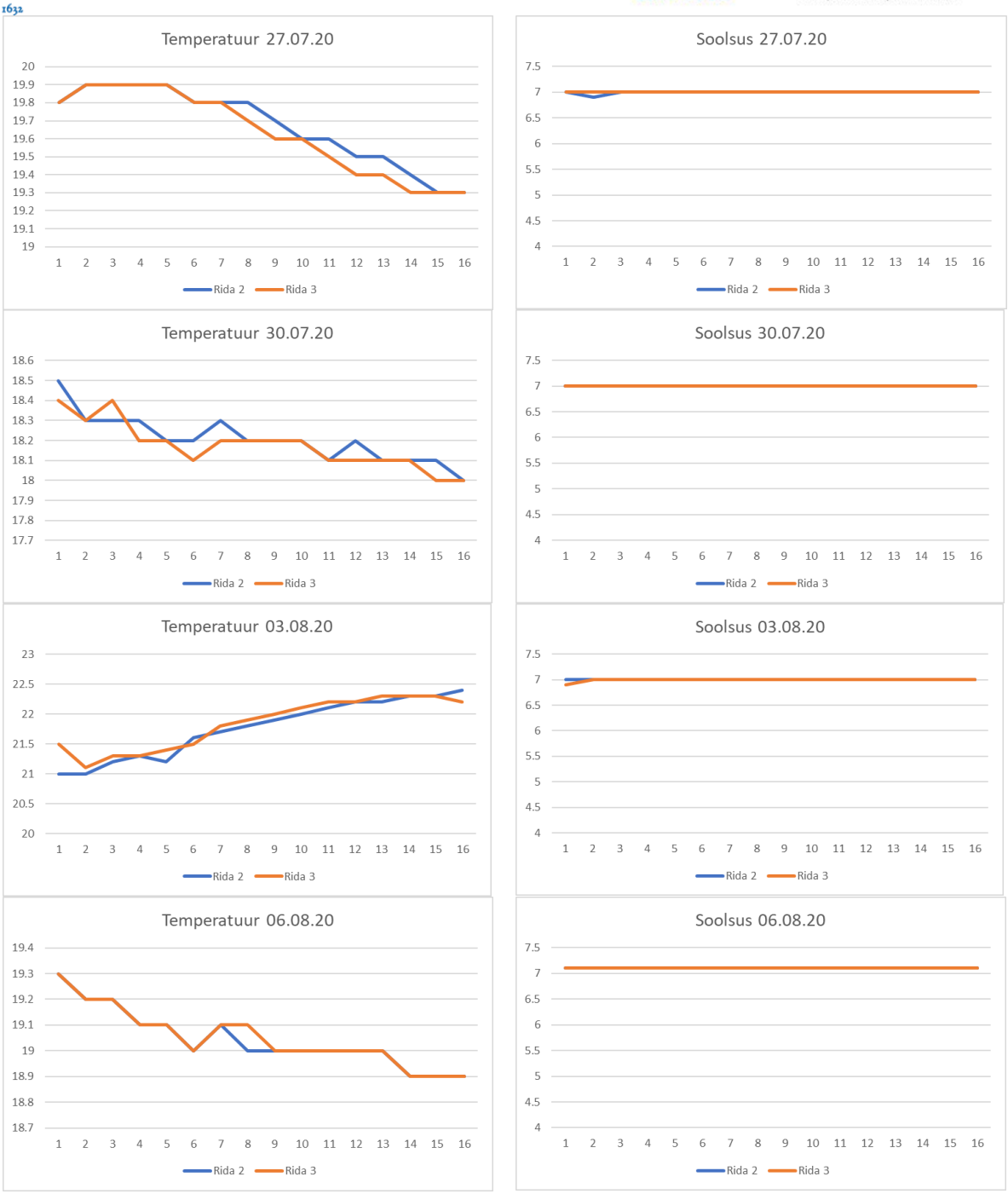
<u>Toitainete ärastamine kalakasvatusveest.</u> Läbiviidud eksperimentide käigus õnnestus saavutada teatud soodsatel tingimustel nitraatide ja nitritite sidumine võrreldes kontrolliga isegi kuni 10 % nitraatide puhul ja 20-25 % nitritide puhul (Joonised 31 ja 32). Andmete suure varieeruvuse tõttu ei saanud statistiliselt olulist erinevust kontrolli ja vetikamahutite vahel fosforiühendite puhul. Samas kogu süsteemi toitainete ärastamise efektiivsus oli äärmiselt kõrge – nitraatide ja nitritite puhul kuni 60 %, fosfaadi puhul 60 % ja üldfosfori puhul 30 % kalamahutist filtersüsteemi tuleva vee kontsentratsioonist (Joonised 31-35).

Vee erikasutusloa seire tulemused näitasid samuti, et kogu süsteem toimis efektiivse filtrina. Võrreldes sissetuleva vee sisaldustega vähenes süsteemist väljavoolavas vees oluliselt nitraatide ja fosfaatide kogus. Võrreldes kalamahuti järgsest jämefiltrist väljuvast vees vähenes süsteemist väljuvas vees oluliselt üldlämmastiku ja üldfosfori kontsentratsioon just neljanda eksperimendi ajal (LISA 4, Joonis 1).

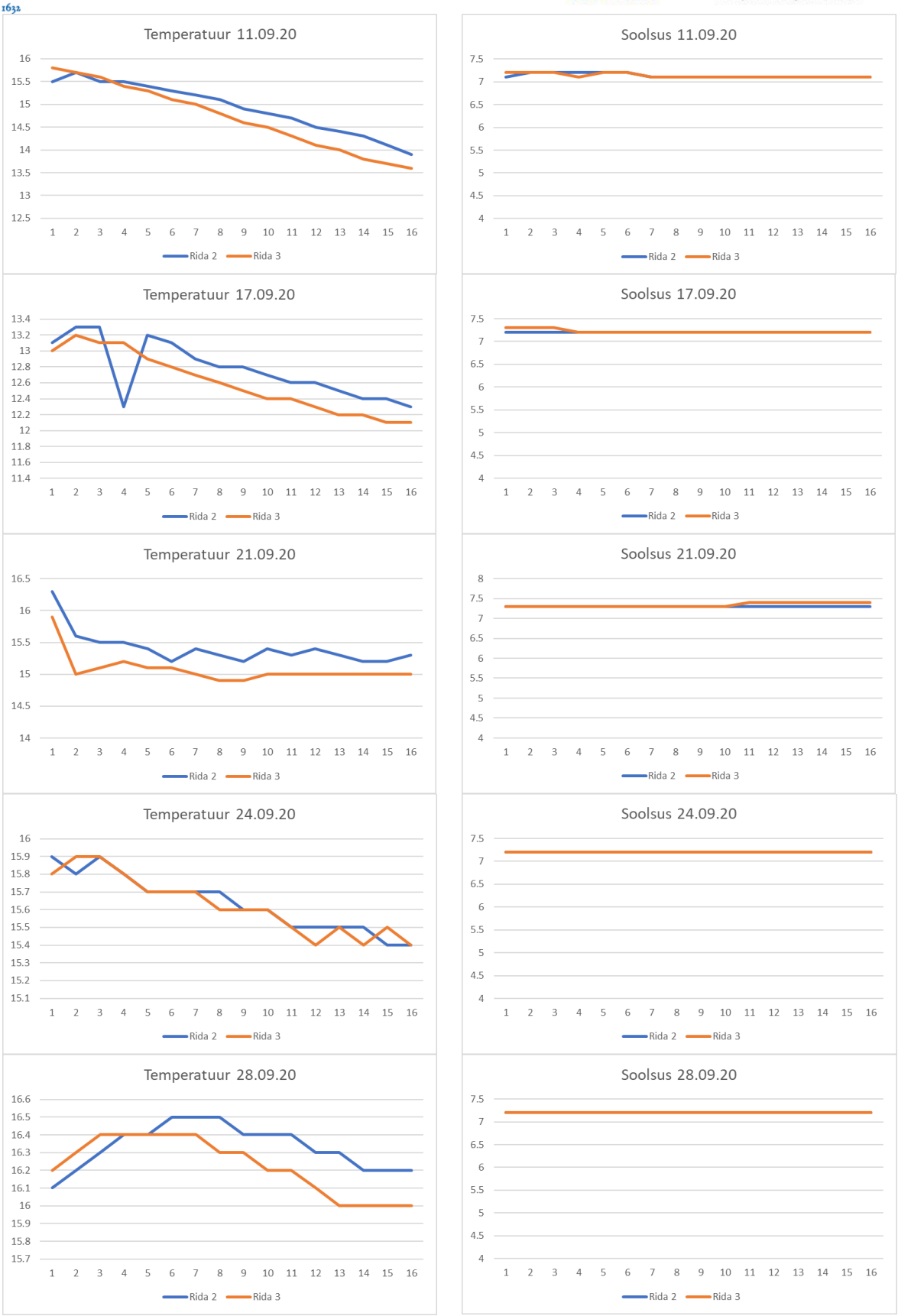
<u>Vetikabiomassi juurdekasv.</u> Vetikabiomassi juurdekasvu määramiseks kaaluti mahutites olev Ulva biomass nii enne kui pärast eksperimenti. Vetikabiomassi juurdekasv oli negatiivne esimese ja teise eksperimendi puhul ja positiivne kolmanda eksperimendi puhul. Kõige suurem oli vetikamassi juurdekasv mahutites 1.4 ja 1.5, kus see ulatus kuni 4 % juurdekasvu päevas (Joonis 36). Suur erinevus erinevate mahutijadade vahel on tingitud tehnilistest probleemidest (veevoolu muutus, vetikamassi kaod, veevoolu ummistused jne.).



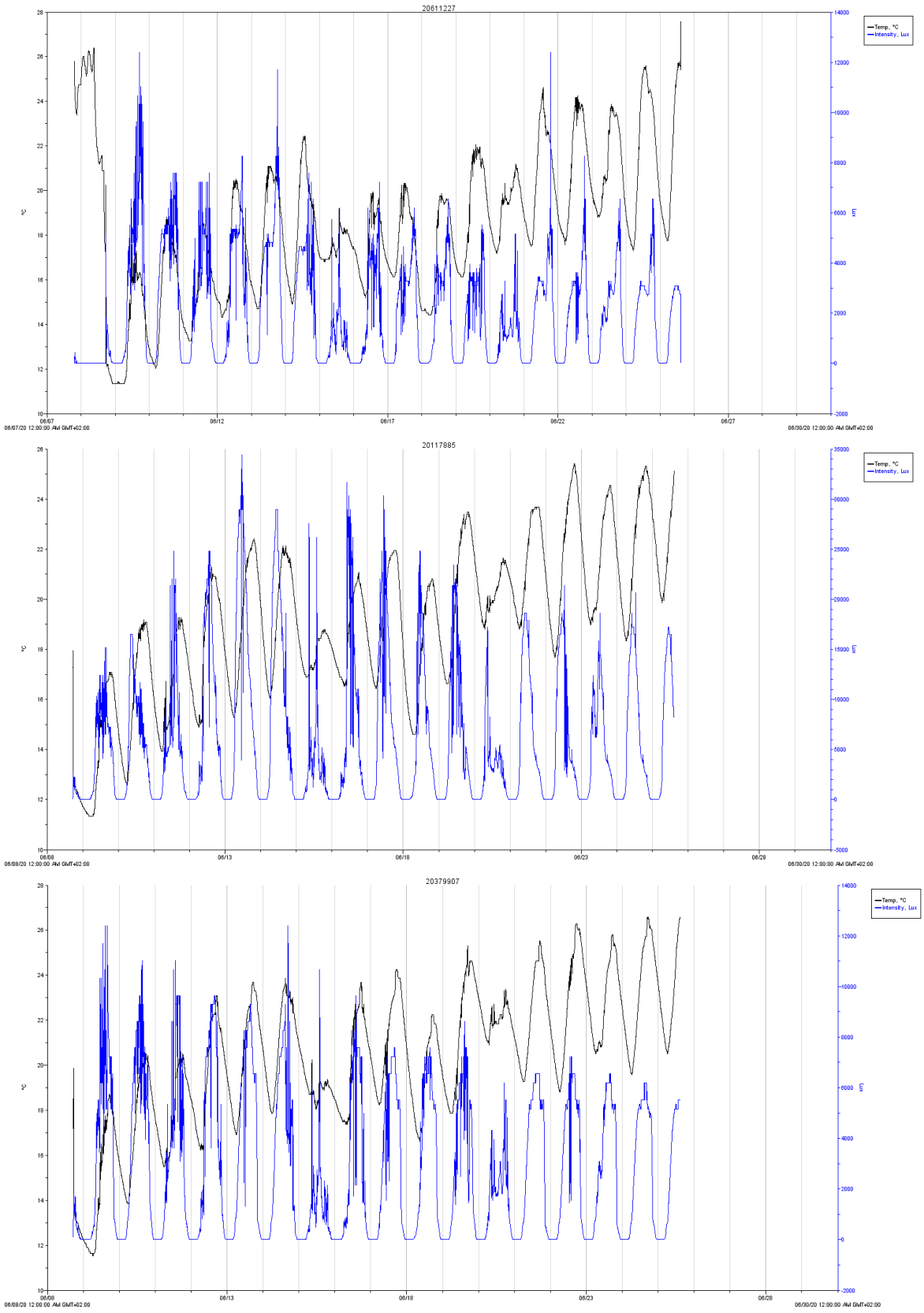
Joonis 27. Soolsus ja temperatuur mahutites 2020. aasta esimese eksperimendi ajal (x - teljel mahutite numbrid, seeriad vastavalt mahutite Rida 2 ja Rida 3).



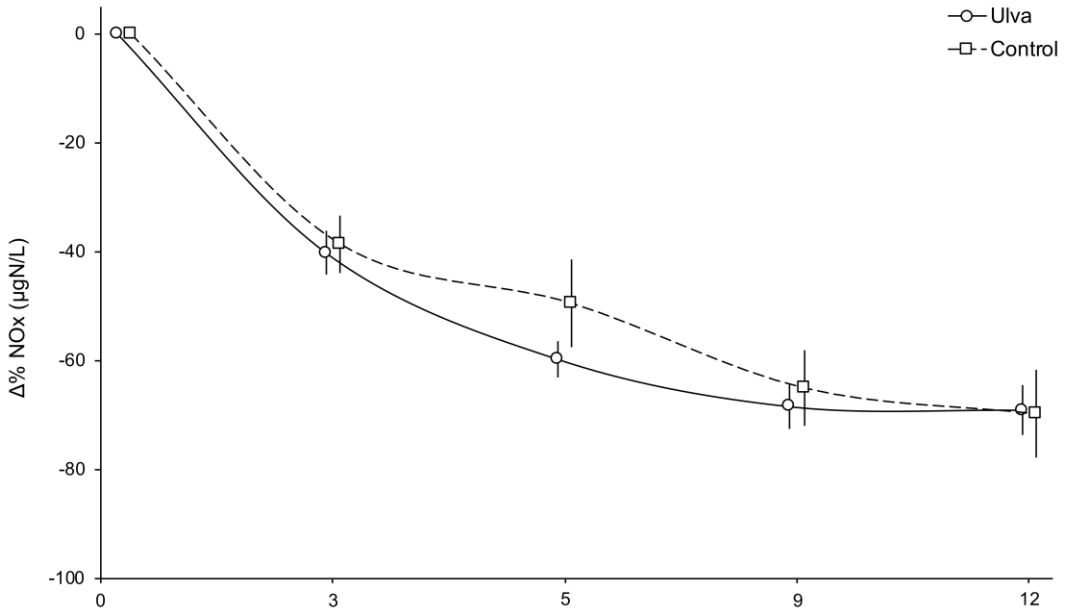
Joonis 28. Soolsus ja temperatuur mahutites 2020. aasta teise eksperimendi ajal (x - teljel mahutite numbrid, seeriad vastavalt mahutite Rida 2 ja Rida 3).



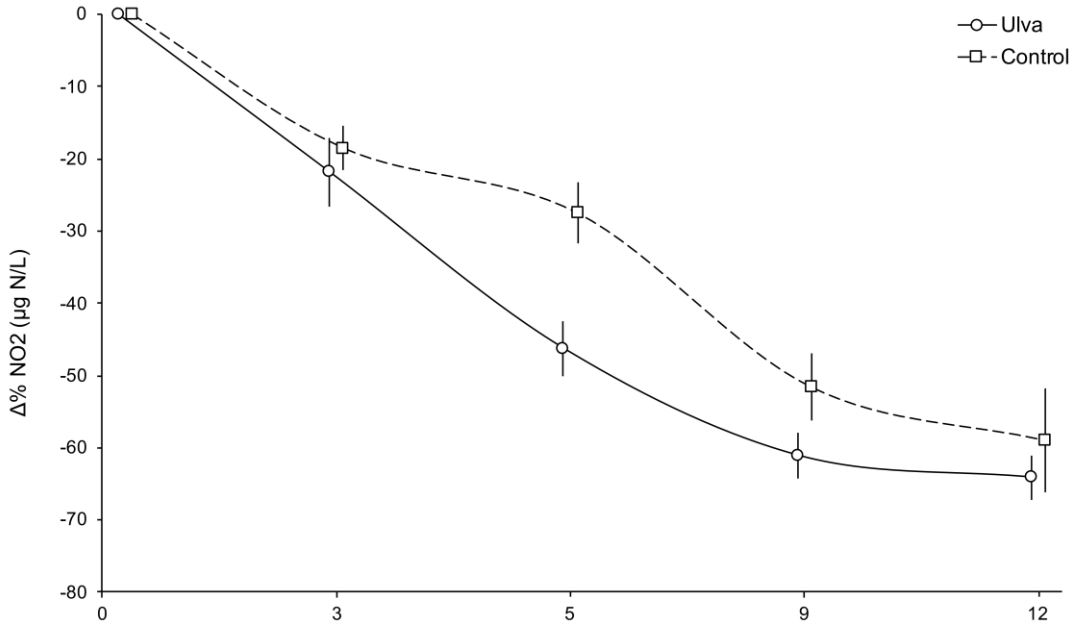
Joonis 29. Soolsus ja temperatuur mahutites 2020. aasta kolmanda eksperimendi ajal (x - teljel mahutite numbrid, seeriad vastavalt mahutite Rida 2 ja Rida 3).



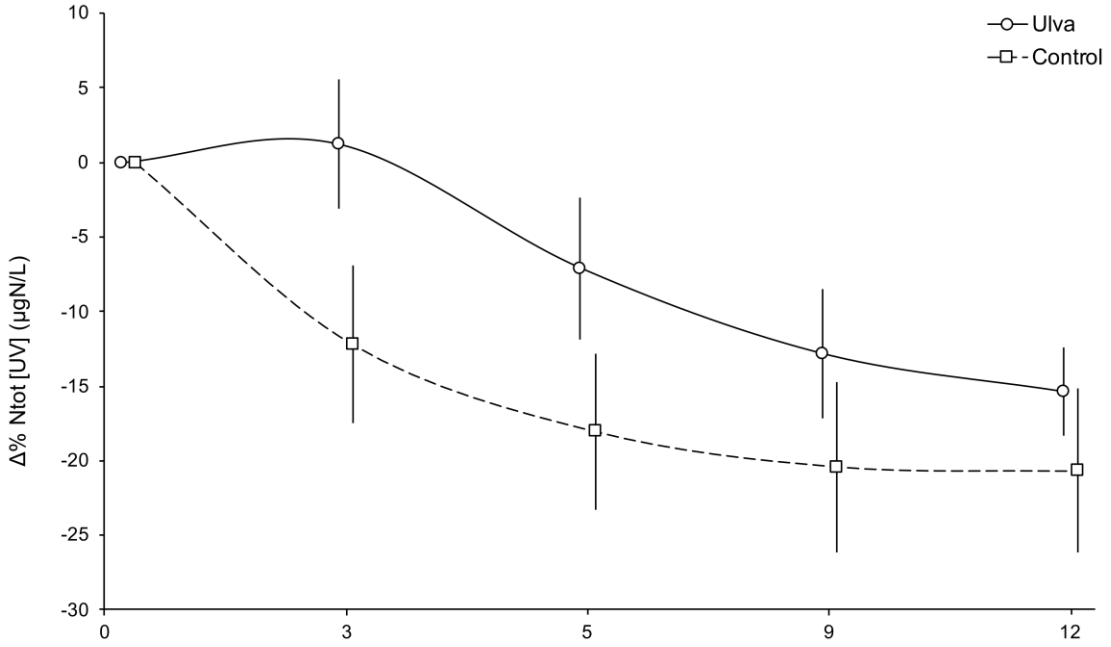
Joonis 30. Valgus- ja temperatuurilogeri näidud 2020. aasta esimese eksperimendi ajal. (Loger 20611227 – mahuti 1.1; loger 20117885 - mahuti 1.6; loger 20379907 – mahuti 1.14).



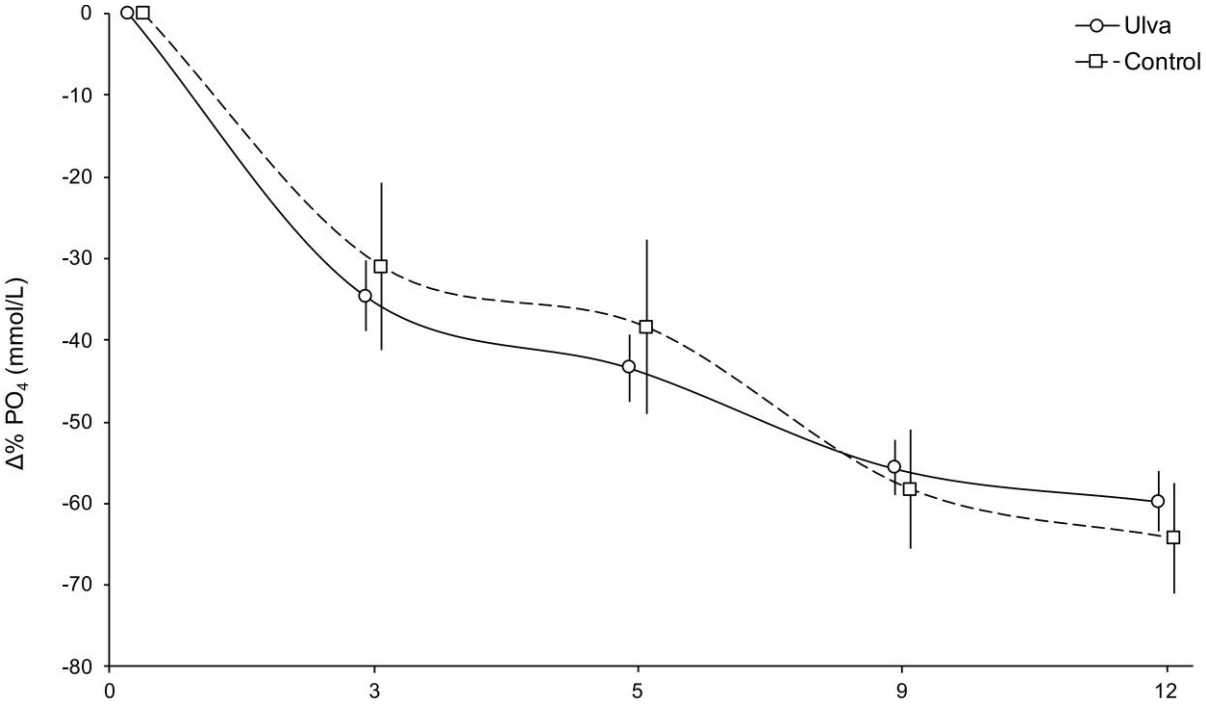
Joonis 31. Lämmastikuühendite kontsentratsiooni muutus biofiltersüsteemis 2020. aasta eksperimentide põhjal (andmetest eemaldatud ilmastiku või tehniliste probleemide tõttu probleemsed mõõtmistulemused).



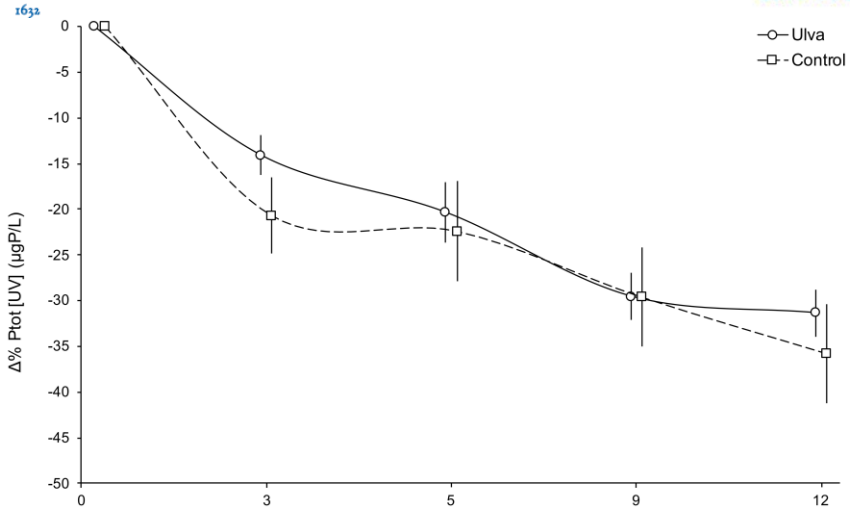
Joonis 32. Nitritite kontsentratsiooni muutus biofiltersüsteemis 2020. aasta eksperimentide põhjal (andmetest eemaldatud ilmastiku või tehniliste probleemide tõttu probleemsed mõõtmistulemused).



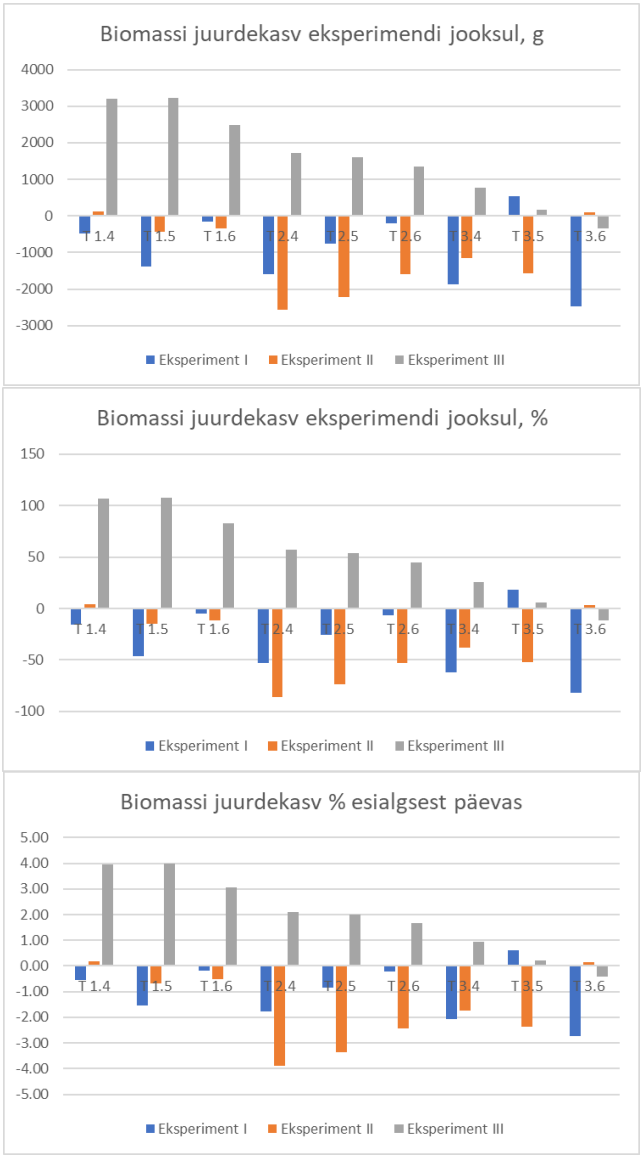
Joonis 33. Üldlämmastiku kontsentratsiooni muutus biofiltersüsteemis 2020. aasta eksperimentide põhjal (andmetest eemaldatud ilmastiku või tehniliste probleemide tõttu probleemsed mõõtmistulemused).



Joonis 34. Fosfaatide kontsentratsiooni muutus biofiltersüsteemis 2020. aasta eksperimentide põhjal (andmetest eemaldatud ilmastiku või tehniliste probleemide tõttu probleemsed mõõtmistulemused).



Joonis 35. Üldfosfori kontsentratsiooni muutus biofiltersüsteemis 2020. a eksperimentide põhjal (andmetest eemaldatud ilmastiku või tehniliste probleemide tõttu probleemsed mõõtmistulemused).



Joonis 36. Vetikabiomassi juurdekasv 2020. aastal läbiviidud eksperimentide käigus.

Joonis 37. Mahutites kultiveeritud *Ulva intestinalis* (september 2019).

Kirjeldamaks mujal maailmas vetikabiomassi kasutamise kogemust koostati käesoleva projekti käigus kirjanduse ülevaade (lisatud käesolevale aruandele, LISA 7). Allpool tuuakse ära selle töö tähtsamad leiud.

Kultiveeritud või rannalt kogutud vetikabiomassi kasutus saab olla ühes järgmistest valdkondadest:

- kompost ja põllumajandusväetis
- põletamine energia saamiseks
- biogaasi tootmine
- biodiisli või bioetanooli tootmine
- tooraine erinevate kasulike bioloogiste ühendite saamiseks
- kalasööda komponentide tootmine
- loomasööda komponentide tootmine
- toiduained inimtarbimiseks

Käesoleva projekti käigus katsetes kõige edukamaks osutunud liik *Ulva intestinalis* sobib kirjanduse põhjal mitme mainitud valdkonna järgseks kasutuseks. Mainitakse nii otsest põletamist kui biogaasi ja biodiisli tootmist. Samuti kasutatakse sarnaseid liike vesiviljeluses kalasööda komponentidena. Viimaste aastate trendiks on ka vetika lisamine loomasööta – paljudel liikidel on avastatud võime vähendada veistel metaani eraldumist sööda seedimisel. *Ulva intestinalis* on üheks potentsiaalseks liigiks kasutamiseks loomasööda komponendina.

Eestis ei ole hetkel kogemust suurel hulgal vetikabiomassi tööndusliikuks kasutamiseks v.a. agariku kasutamine. Käesolevas töös oli põhieesmärk saavutada kalakasvatuse heitvee toitainete sisalduse vähenemine vetikaid sisaldava biofiltersüsteemi abil. Biofiltersüsteemi jaoks sobivad vetikaliigid peavad olema väga kiire kasvuga, see tagab efektiivse toitainete sidumise. Agarik selleks ei sobi. Samas suur toitainete omastamise määr tähendab ka suurt biomassi juurdekasvu. Selle biomassi utiliseerimine võib olla suure tootmisvõimsusega kalakasvatuse puhul suureks probleemiks ja seepärast oleks otstarbekas leida sellele vetikabiomassile rakendus. Konkreetse lahenduse leidmine sõltub konkreetse projekti kontekstist ja äriplaanist.

Ohtlike ainete sisaldus mahutis kasvatatud vetikamassis.

Veendumaks mahutites kasvatatava vetikamassi ohutuses ja sobivuses kasutamiseks kas inimese toiduks või sööda tootmiseks, teostati raskemetallide analüüsid inkubeeritud vetikamassist kahel korral erinevate inkubeerimiseksperimentide käigus (analüüside akt lisas, LISA 6).

EL on kehtestanud ohtlike ainete piirnormide väärtused söötades (2002/32/EÜ). Seejuures on kasutatud erinevaid mõisteid. Vetikatega seoses võib nendest välja tuua kaks peamist:

“Söödad” – loomade söötmiseks mõeldud töötlemata, värsked või konserveeritud taimset või loomset päritolu tooted, nendest tööstusliku töötlemise abil saadud tooted ning eraldi või segudena kasutatavad orgaanilised või anorgaanilised ained, lisanditega või lisanditeta;

“Söödamaterjalid” – mitmesugused töötlemata, värsked või konserveeritud taimset või loomset päritolu tooted, nendest tööstusliku töötlemise abil saadud tooted ning eraldi või segudena

kasutatavad orgaanilised või anorgaanilised ained, lisanditega või lisanditeta, söötmiseks loomadele kas töötlemata kujul, töötlemise abil saadud segasöötade koostises või eelsegude substraatidena. (2002/32/EÜ)

Piirnormide väärtused (piirnorm (mg/kg (ppm)) sööda puhul, mille niiskusesisaldus on 12 %) erinevates materjalides on toodud nelja metalli kohta – arseen, plii, elavhõbe ja kaadmium.

Arseen. Söödamaterjal – 2 mg/kg (2002/32/EÜ) ; fosfaadid ja lubjarikkad merevetikad – 10 mg/kg); merevetikajahu ja merevetikatest valmistatud söödamaterjal – 40 mg/kg (2012/744/EL). Sisaldused analüüsitud vetikates on võrreldavad söödamaterjalide kohta toodud piirnormidega, kuid oluliselt madalamad merevetikate kohta toodud piirnormidest.

Plii. Söödamaterjal - 10 mg/kg (2002/32/EÜ); haljassööt – 30 mg/kg; fosfaadid ja lubjarikkad merevetikad, 15 mg/kg (2012/744/EL; 2013/1275/EL). Jättes kõrvale ühe erakordselt kõrge plii sisaldusega proovi (TA21000558), on plii sisaldused vetikates madalamad kehtestatud piirnormidest.

Elavhõbe. Söödamaterjal – 0,1 mg/kg (2002/32/EÜ). Kõigis analüüsitud vetikaproovides on elavhõbeda sisaldus piirnormist madalam.

Kaadmium. Taimse päritoluga söödatooraine – 1 mg/kg (2002/32/EÜ). Kõigis analüüsitud vetikaproovides on kaadmiumi sisaldus oluliselt madalam piirnormi väärtusest.

Soovitused tehnoloogia rakendamiseks

Suurvetikatel põhinev biofiltersüsteem kalakasvatuse vee puhastamiseks on reaalne alternatiiv muudele kalakasvatuse heitvee töötlemise võimalustele. Meie katsed näitasid, et teatud parimatel tingimustel saab nii saavutada väga suure toitainete eemaldamise efektiivsuse ja olulise vetikabiomassi juurdekasvu. Samas on vajalik tehnoloogia edasine täiustamine kuna käesoleva projekti formaat ei võimaldanud oluliselt muuta juba algselt kavandatud tööplaani ja katse tehnilist lahendust.

Sarnase tehnoloogia rakendamisel on oluline arvestada järgmiste kogemustega, mis tekkisid projekti läbiviimisel:

1. Inkubatsioonimahutite kuju – projektis kasutatud inkubatsioonimahutid osutusid ebamugavaks saavutamaks kõige optimaalsemaid vetikabiomassi inkubeerimise tingimusi. Inkubatsioonimahutid peavad võimaldama läbivoolava vee vaba, takistusteta voolu läbi süsteemi ja on oluline fikseerida vabalt hõljuv vetikamass mahutites. Meie juhul kasutasime 2020. aasta eksperimentide puhul vetikamassi fikseerimiseks suure silmaga nailonvõrku. Suurimaks probleemiks oli meie eksperimendi puhul mahuteid ühendavate veetorude ummistused vetikamassiga. Kuna projektis esialgselt kavandatud mahuteid ei olnud võimalik hankida, kasutati lihtsustatud modulaarset mahutite versiooni. Optimaalsem oleks kasutada madalamaid, suuremate mõõtmetega, parema läbivooluga mahuteid.
2. Ilmastiku mõju - veetemperatuur on äärmiselt oluline keskkonnategur, mis mõjutab kõikide veeorganismide füsioloogiat. Avatud basseinide või mahutite süsteem on ilmastikutingimuste meelevallas ja selliste süsteemide puhul on raske ära hoida ekstreemsete tingimuste tekkimist. Selliste vetikakasvatuse mahutite kasutamisel peab olema läbi mõeldud ka kaitse ekstreemsete ilmastikutingimuste eest (kaitse ülemäärase päikesekiirguse eest).
3. Vetikaliigi valik – biofiltersüsteemi vetikaliigi valiku puhul tuleks arvestada päris mitme teguriga. Eelkõige peaks see olema kohalik liik. Liik peaks olema kõrge biomassi

produktsiooniga – toitainete sidumisvõimega. Liik peaks olema kultiveeritav substraadile kinnitamata. Liik peaks olema pika vegetatsiooniperioodiga – võimaldama biofiltersüsteemi kasutamist võimalikult pika hooaja jooksul. Meie poolt kasutatud *Ulva intestinalis* on väga perspektiivne liik kultiveerimiseks just kalakasvatuse heitveest toitainete eemaldajana.

4. Kultiveerimise alustamiseks vajaliku vetikakoguse hankimine – oluliseks probleemiks kirjeldatud suurvetikatel põhineva filtersüsteemi käivitamisel võib kujuneda esialgse vetikabiomassi hankimine. Kuna seda hetkel ei ole võimalik tööndulikes mahtudes soetada ei Eestis ega ka lähipiirkonnas siis vajab vetikainkubaatori käivitamiseks piisava koguse toormaterjali kogumine loodusest eraldi korraldamist. Hiljem, süsteemi regulaarsel käitamisel saab kasutada varasemal perioodil genereeritud biomassi, samas tuleb vetikabiomassi ka uuendada. Käesolevas projektis (lühiajalised eksperimendid) sellist kogemust ei tekkinud.
5. Vetikainkubaatori suurus – vetikainkubaatori suurus sõltub paljudest teguritest nagu kultiveeritava kala kogusest, veevoolu hulgast, soovitud toitainete ärastamise efektiivsusest, soovitud kultiveeritud vetikabiomassi suurusest jne. Meie eksperimentide puhul oli kogu süsteem veidi üledimensioneeritud – veevool liiga suur, kalade kogus liiga väike.
6. Jämesette eemaldamine – väga oluline on kalamahutist tuleva vee puhastamine jämedast settest enne vetikainkubaatorisse suunamist, kuna jämesete sattudes kultiveeritava vetika hulka vähendab vetikabiomassi kvaliteeti ja põhjustab muude liikide vohamise.
7. Keskkonnaparameetrite pidev seire – äärmiselt oluline on jälgida kogu süsteemi keskkonnaparameetreid, et operatiivselt reageerida tekkivatele olukordadele. Meie projekti kogemus näitas, et isegi päevane hilinemine olukorrale võib põhjustada kogu süsteemi seiskumise ja inkubeeritava vetikabiomassi hukkumise.
8. Regulaarne vetikabiomassi eemaldamine – juhul, kui vetikabiomass kasvab optimaalsetes tingimustes on tema lisandumise kiirus väga suur. Seega on vajalik regulaarne vetikabiomassi koguse reguleerimine mahutites.

English summary

The current project was designed to assess the feasibility of using macroalgae as a biological filtration system for the removal of dissolved nutrients found in seawater based fish farm effluent/waste water. To test this, an experimental fish farm was established on the northern coast of Saaremaa island, West Estonian archipelago. The experimental fish farm setup consisted of a 6 m^3 fish tank stocked with finfish, a water pump station, aeration devices and mechanical filter units connected to a series of 0,8m^3 tanks used for the incubation of macroalgae.

The study was conducted in several phases – firstly, suitable macroalgae candidates were selected from species found to grow naturally within the local coastal region. Independent experiments were conducted in order to assess each species ability to uptake nutrients and as consequence its utility as a biofilter. Of these, the most suitable candidate for use in a biofiltration system was identified to be the green algae *Ulva intestinalis*. In the second phase, one vegetative season (2020) was dedicated to testing the efficiency of nutrient removal by a filter system inhabited by *Ulva intestinalis*. In total, three, 4-5 week experiments were conducted in which fish farm waste water outflow was monitored in terms of parameters relating to biotic and abiotic factors, with farm external environmental factors also monitored.

Whilst all three experiments experienced differences in environmental settings and a very high variability in response variables observed, we managed to obtain a 10-15 % reduction in waste water nutrient concentrations (primarily nitrogenous compounds) for tanks inhabited by algae compared to that of control tanks. As a whole, the system experienced an average 60% reduction in nitrogen and phosphorus concentrations in waste water outflow compared to concentrations present within the fish tank. Additionally, the subsequent biomass gain of the incubated macroalgae species *Ulva intestinalis* is reported to be 4% per day at its maximum rate.

Overall, the results obtained from this study indicate a promising future for the development of biological filtration setups. The implantation of this technology as an alternative to mechanical and other biological methods of treatment for fish farm effluents may offer both economic and environmental benefits for existing and future finfish aquaculture operations.

Kasutatud kirjandus

2002/32/EÜ EUROOPA PARLAMENDI JA NÕUKOGU DIREKTIIV, 7. mai 2002, loomatoidus leiduvate soovimatute ainete kohta.

2012/744/EL KOMISJONI MÄÄRUS, 16. august 2012, millega muudetakse Euroopa Parlamendi ja nõukogu direktiivi 2002/32/EÜ I ja II lisa arseeni, fluori, plii, elavhõbeda, endosulfaani, dioksiinide, Ambrosia spp., diklasuriili ja naatriumlasalotsiid A piirnormide ja dioksiinide rakenduskünniste osas.

2013/1275/EL KOMISJONI MÄÄRUS, 6. detsember 2013, millega muudetakse Euroopa Parlamendi ja nõukogu direktiivi 2002/32/EÜ I lisa arseeni, kaadmiumi, plii, nitritite, lenduva sinepiõli ja kahjulike botaaniliste lisandite osas

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LISA 1

Tabel 1. 2018. aasta veekeemia analüüside tulemused.

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
Kesknõmme kalatünn	29/11/2018	0.72	0.85	4.73	0.92	29.50
Kesknõmme kalatünn	29/11/2018	0.79	0.79	4.86	0.96	32.65
Kesknõmme kalatünn	29/11/2018	0.84	0.77	4.66	0.81	29.07
Kesknõmme meri	29/11/2018	0.69	0.69	5.41	0.76	26.07
Kesknõmme meri	29/11/2018	0.65	0.72	5.10	0.74	25.04
Kesknõmme meri	29/11/2018	0.75	0.70	4.27	0.78	21.26
Kesknõmme tünn 1	29/11/2018	0.60	0.88	3.26	0.88	25.05
Kesknõmme tünn 1	29/11/2018	0.87	1.03	4.88	1.10	33.01
Kesknõmme tünn 1	29/11/2018	0.88	1.06	4.83	1.18	34.63
Kesknõmme tünn 2	29/11/2018	0.69	1.27	3.55	1.32	35.93
Kesknõmme tünn 2	29/11/2018	0.79	1.31	4.98	1.38	36.77
Kesknõmme tünn 2	29/11/2018	0.76	1.44	4.69	1.43	50.25
Kesknõmme tünn 3	29/11/2018	1.01	1.44	4.87	1.58	45.14
Kesknõmme tünn 3	29/11/2018	0.80	1.34	4.91	1.43	36.41
Kesknõmme tünn 3	29/11/2018	0.90	1.42	4.83	1.48	38.25
Kesknõmme tünn 4	29/11/2018	0.79	1.03	4.69	1.08	34.41
Kesknõmme tünn 4	29/11/2018	0.87	1.02	4.83	1.06	32.03
Kesknõmme tünn 4	29/11/2018	0.59	1.08	4.36	1.14	32.38
Kesknõmme kalatünn	09/12/2018	0.94	0.77	6.40	0.82	28.40
Kesknõmme kalatünn	09/12/2018	1.01	0.72	6.61	0.71	28.18
Kesknõmme kalatünn	09/12/2018	1.01	0.75	6.49	0.75	30.62
Kesknõmme meri	09/12/2018	1.00	0.96	6.67	0.96	31.21
Kesknõmme meri	09/12/2018	1.00	0.96	6.54	1.01	33.20
Kesknõmme meri	09/12/2018	0.77	0.80	4.82	0.81	24.25
Kesknõmme tünn 1	09/12/2018	0.99	0.80	6.42	0.84	30.82
Kesknõmme tünn 1	09/12/2018	0.98	0.76	6.38	0.81	29.06
Kesknõmme tünn 1	09/12/2018	0.83	0.76	6.36	0.73	30.02
Kesknõmme tünn 2	09/12/2018	1.01	0.77	6.53	0.69	30.43
Kesknõmme tünn 2	09/12/2018	1.01	0.76	6.38	0.79	29.35
Kesknõmme tünn 2	09/12/2018	0.45	0.59	5.07	0.61	22.56
Kesknõmme tünn 3	09/12/2018	0.96	0.74	6.68	0.77	28.79
Kesknõmme tünn 3	09/12/2018	0.61	0.60	4.39	0.66	20.76
Kesknõmme tünn 3	09/12/2018	0.98	0.76	6.45	0.80	28.87
Kesknõmme tünn 4	09/12/2018	0.95	0.76	6.34	0.75	28.78
Kesknõmme tünn 4	09/12/2018	0.96	0.71	6.44	0.76	28.82
Kesknõmme tünn 4	09/12/2018	0.97	0.73	6.28	0.78	28.67

Tabel 2. 2019. aasta veekeemia analüüside tulemused.

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
T0	25/04/2019	0.72	0.85	4.73	0.92	29.50
T0	25/04/2019	0.79	0.79	4.86	0.96	32.65
T0	25/04/2019	0.84	0.77	4.66	0.81	29.07
T 1.2	25/04/2019	0.69	0.69	5.41	0.76	26.07
T 1.4	25/04/2019	0.65	0.72	5.10	0.74	25.04
T 1.7	25/04/2019	0.75	0.70	4.27	0.78	21.26
T 1.12	25/04/2019	0.60	0.88	3.26	0.88	25.05
T 1.16	25/04/2019	0.87	1.03	4.88	1.10	33.01
T 2.2	25/04/2019	0.88	1.06	4.83	1.18	34.63
T 2.4	25/04/2019	0.69	1.27	3.55	1.32	35.93
T 2.7	25/04/2019	0.79	1.31	4.98	1.38	36.77
T 2.12	25/04/2019	0.76	1.44	4.69	1.43	50.25
T 2.16	25/04/2019	1.01	1.44	4.87	1.58	45.14
T 3.2	25/04/2019	0.80	1.34	4.91	1.43	36.41
T 3.4	25/04/2019	0.90	1.42	4.83	1.48	38.25
T 3.7	25/04/2019	0.79	1.03	4.69	1.08	34.41
T 3.12	25/04/2019	0.87	1.02	4.83	1.06	32.03
T 3.16	25/04/2019	0.59	1.08	4.36	1.14	32.38
T 4.2	25/04/2019	0.94	0.77	6.40	0.82	28.40
T 4.4	25/04/2019	1.01	0.72	6.61	0.71	28.18
T 4.7	25/04/2019	1.01	0.75	6.49	0.75	30.62
T 4.12	25/04/2019	1.00	0.96	6.67	0.96	31.21
T 4.16	25/04/2019	1.00	0.96	6.54	1.01	33.20
T0	02/05/2019	0.77	0.80	4.82	0.81	24.25
T0	02/05/2019	0.99	0.80	6.42	0.84	30.82
T0	02/05/2019	0.98	0.76	6.38	0.81	29.06
T 1.2	02/05/2019	0.83	0.76	6.36	0.73	30.02
T 1.4	02/05/2019	1.01	0.77	6.53	0.69	30.43
T 1.8	02/05/2019	1.01	0.76	6.38	0.79	29.35
T 1.12	02/05/2019	0.45	0.59	5.07	0.61	22.56
T 1.16	02/05/2019	0.96	0.74	6.68	0.77	28.79
T 2.2	02/05/2019	0.61	0.60	4.39	0.66	20.76
T 2.4	02/05/2019	0.98	0.76	6.45	0.80	28.87
T 2.8	02/05/2019	0.95	0.76	6.34	0.75	28.78
T 2.12	02/05/2019	0.96	0.71	6.44	0.76	28.82
T 2.16	02/05/2019	0.97	0.73	6.28	0.78	28.67
T 3.2	02/05/2019	0.04	0.12	0.47	0.41	27.05
T 3.4	02/05/2019	0.02	0.12	0.33	0.48	25.63
T 3.8	02/05/2019	0.01	0.12	0.30	0.42	21.25
T 3.12	02/05/2019	0.02	0.11	0.18	0.44	17.62
T 3.16	02/05/2019	0.02	0.19	0.30	0.46	18.18
T 4.2	02/05/2019	0.02	0.21	0.30	0.50	21.57

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
T 4.4	02/05/2019	0.02	0.16	0.26	0.45	17.56
T 4.8	02/05/2019	0.02	0.16	0.30	0.47	21.10
T 4.12	02/05/2019	0.02	0.16	0.34	0.39	14.54
T 4.16	02/05/2019	0.02	0.13	0.30	0.48	24.02
T0	17/05/2019	0.02	0.18	0.30	0.53	22.58
T0	17/05/2019	0.02	0.20	0.27	0.48	19.44
T0	17/05/2019	0.02	0.16	0.26	0.48	16.60
T 1.2	17/05/2019	0.02	0.21	0.31	0.51	20.61
T 1.4	17/05/2019	0.03	0.17	0.55	0.44	18.94
T 1.8	17/05/2019	0.04	0.18	0.30	0.49	19.61
T 1.12	17/05/2019	0.02	0.21	0.28	0.53	21.11
T 1.16	17/05/2019	0.02	0.18	0.38	0.53	20.33
T 2.2	17/05/2019	0.02	0.19	0.42	0.52	19.23
T 2.4	17/05/2019	0.02	0.20	0.25	0.54	19.92
T 2.8	17/05/2019	0.01	0.16	0.20	0.48	16.09
T 2.12	17/05/2019	0.02	0.13	0.16	0.43	15.27
T 2.16	17/05/2019	0.01	0.16	0.15	0.50	16.70
T 3.2	17/05/2019	0.05	0.36	0.69	1.42	23.35
T 3.4	17/05/2019	0.03	0.33	0.67	1.40	22.04
T 3.8	17/05/2019	0.03	0.32	0.63	1.37	22.50
T 3.12	17/05/2019	0.02	0.30	0.52	0.86	17.47
T 3.16	17/05/2019	0.03	0.35	0.49	1.05	19.90
T 4.2	17/05/2019	0.03	0.35	0.57	1.27	21.10
T 4.4	17/05/2019	0.03	0.27	0.55	1.00	19.00
T 4.8	17/05/2019	0.02	0.26	0.41	1.12	19.80
T 4.12	17/05/2019	0.03	0.35	0.64	1.34	22.20
T 4.16	17/05/2019	0.04	0.40	0.63	1.42	22.31
T0	25/05/2019	0.04	0.40	0.57	1.48	22.86
T0	25/05/2019	0.04	0.34	0.63	1.45	22.86
T0	25/05/2019	0.01	0.30	0.42	0.98	18.43
T1.2	25/05/2019	0.03	0.34	0.64	1.38	22.39
T1.4	25/05/2019	0.04	0.43	0.58	1.44	23.54
T1.8	25/05/2019	0.04	0.38	0.56	1.45	23.85
T1.12	25/05/2019	0.04	0.29	0.59	1.28	20.14
T1.16	25/05/2019	0.02	0.30	0.62	0.89	16.95
T2.2	25/05/2019	0.03	0.34	0.63	1.46	21.35
T2.4	25/05/2019	0.05	0.35	0.64	1.34	21.74
T2.8	25/05/2019	0.03	0.35	0.43	1.08	19.19
T2.12	25/05/2019	0.02	0.37	0.27	1.48	22.90
T2.16	25/05/2019	0.02	0.29	0.22	1.35	20.12
T3.2	25/05/2019	0.02	0.10	0.15	0.59	28.64
T3.4	25/05/2019	0.02	0.10	0.34	0.69	45.39
T3.8	25/05/2019	0.02	0.11	0.29	0.59	26.90
T3.12	25/05/2019	0.02	0.15	0.23	0.59	25.77

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
T3.16	25/05/2019	0.02	0.19	0.27	0.58	23.33
T4.2	25/05/2019	0.02	0.19	0.20	0.57	22.10
T4.4	25/05/2019	0.02	0.18	0.19	0.57	18.67
T4.8	25/05/2019	0.01	0.13	0.19	0.55	22.34
T4.12	25/05/2019	0.02	0.20	0.30	0.57	23.27
T4.16	25/05/2019	0.03	0.18	0.27	0.54	21.90
T0	04/06/2019	0.02	0.19	0.24	0.61	22.17
T0	04/06/2019	0.02	0.19	0.17	0.63	19.68
T0	04/06/2019	0.02	0.21	0.39	0.60	21.90
T1.2	04/06/2019	0.03	0.15	0.31	0.52	24.11
T1.4	04/06/2019	0.01	0.19	0.28	0.55	22.33
T1.8	04/06/2019	0.01	0.15	0.25	0.51	19.25
T1.12	04/06/2019	0.02	0.18	0.22	0.57	21.64
T1.16	04/06/2019	0.01	0.17	0.29	0.69	24.94
T2.2	04/06/2019	0.01	0.12	0.33	0.56	30.99
T2.4	04/06/2019	0.02	0.10	0.28	0.51	26.51
T2.8	04/06/2019	0.02	0.17	0.22	0.54	21.58
T2.12	04/06/2019	0.01	0.14	0.22	0.49	21.60
T2.16	04/06/2019	0.01	0.14	0.21	0.51	22.08
T3.2	04/06/2019	0.02	0.29	0.27	0.74	22.70
T3.4	04/06/2019	0.02	0.25	0.21	0.89	27.71
T3.8	04/06/2019	0.02	0.27	0.22	0.73	24.82
T3.12	04/06/2019	0.02	0.34	0.22	0.87	23.98
T3.16	04/06/2019	0.04	0.23	0.37	0.65	23.23
T4.2	04/06/2019	0.02	0.15	0.22	0.59	22.44
T4.4	04/06/2019	0.01	0.15	0.29	0.52	22.05
T4.12	04/06/2019	0.01	0.13	0.35	0.48	23.16
T4.16	04/06/2019	0.02	0.32	0.24	0.75	23.20
T0	18/06/2019	0.03	0.24	0.50	0.66	24.10
T0	18/06/2019	0.02	0.16	0.22	0.50	21.40
T0	18/06/2019	0.02	0.15	0.26	0.49	23.33
T1.2	18/06/2019	0.01	0.11	0.21	0.43	19.30
T1.4	18/06/2019	0.03	0.35	0.24	0.84	24.21
T1.8	18/06/2019	0.03	0.27	0.48	0.63	22.57
T1.12	18/06/2019	0.02	0.16	0.26	0.57	23.09
T1.16	18/06/2019	0.01	0.15	0.32	0.48	18.43
T2.2	18/06/2019	0.02	0.16	0.27	0.52	23.38
T2.4	18/06/2019	0.02	0.20	0.20	0.61	21.47
T2.8	18/06/2019	0.01	0.25	0.27	0.72	30.32
T2.12	18/06/2019	-0.01	0.18	0.24	0.52	24.70
T2.16	18/06/2019	0.00	0.16	0.23	0.38	13.64
T3.2	18/06/2019	0.02	0.11	0.23	0.46	21.71
T3.4	18/06/2019	0.03	0.29	0.32	1.47	27.49
T3.8	18/06/2019	0.03	0.25	0.29	1.36	24.86

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
T3.12	18/06/2019	0.03	0.28	0.29	1.42	24.64
T3.16	18/06/2019	0.03	0.25	0.25	1.54	23.01
T4.2	18/06/2019	0.03	0.24	0.22	1.47	24.46
T4.4	18/06/2019	0.02	0.13	0.22	0.77	19.70
T4.8	18/06/2019	0.02	0.12	0.27	1.30	25.47
T4.12	18/06/2019	0.03	0.14	0.22	1.30	23.15
T4.16	18/06/2019	0.02	0.47	0.21	1.32	22.33
T0	30/07/2019	0.02	0.21	0.23	1.33	23.56
T1.2	30/07/2019	0.01	0.16	0.22	0.86	19.09
T1.4	30/07/2019	0.01	0.13	0.20	1.23	19.90
T1.8	30/07/2019	0.01	0.14	0.28	1.01	19.75
T1.12	30/07/2019	0.03	0.33	0.26	1.57	25.68
T1.16	30/07/2019	0.02	0.21	0.27	0.93	20.60
T2.2	30/07/2019	0.02	0.16	0.25	1.26	24.41
T2.4	30/07/2019	0.03	0.13	0.23	1.23	23.70
T2.8	30/07/2019	0.02	0.12	0.25	1.17	25.05
T2.12	30/07/2019	0.02	0.41	0.23	1.61	25.99
T2.16	30/07/2019	0.02	0.23	0.22	1.24	23.25
T3.2	30/07/2019	0.02	0.18	0.30	1.29	25.29
T3.4	30/07/2019	0.02	0.20	0.22	1.10	20.70
T3.8	30/07/2019	0.05	0.24	0.29	1.32	23.82
T3.12	30/07/2019	0.04	0.25	0.31	1.33	23.65
T3.16	30/07/2019	0.04	0.23	0.33	1.31	24.11
T4.2	30/07/2019	0.07	0.28	0.38	1.40	28.63
T4.4	30/07/2019	0.10	0.31	0.35	1.45	29.17
T4.8	30/07/2019	0.03	0.40	0.28	1.48	30.11
T4.12	30/07/2019	0.12	0.27	0.24	1.48	29.23
T4.16	30/07/2019	0.02	0.30	0.34	1.32	28.56
T0	08/08/2019	0.10	0.41	0.40	1.65	27.36
T0	08/08/2019	0.08	0.43	0.38	1.52	28.33
T0	08/08/2019	0.02	0.29	0.20	1.43	26.96
T1.2	08/08/2019	0.03	0.28	0.19	1.32	25.11
T1.4	08/08/2019	0.02	0.20	0.20	1.20	25.46
T1.8	08/08/2019	0.06	0.48	0.53	1.61	30.11
T1.12	08/08/2019	0.07	0.40	0.38	1.45	29.28
T1.16	08/08/2019	0.03	0.36	0.26	1.38	28.95
T2.2	08/08/2019	0.02	0.25	0.21	1.23	27.15
T2.4	08/08/2019	0.04	0.24	0.25	1.43	27.54
T2.8	08/08/2019	0.04	0.22	0.23	1.26	25.27
T2.12	08/08/2019	0.05	0.29	0.20	1.55	25.16
T2.16	08/08/2019	0.03	0.20	0.22	1.16	24.94
T3.2	08/08/2019	0.04	0.19	0.23	1.14	24.93
T3.4	08/08/2019	0.03	0.18	0.22	1.18	24.48
T3.8	08/08/2019	0.08	0.33	0.40	1.74	33.82

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
T3.12	08/08/2019	0.07	0.15	0.39	1.55	41.22
T3.16	08/08/2019	0.08	0.26	0.42	1.73	36.57
T4.2	08/08/2019	0.06	0.20	0.34	1.77	35.20
T4.4	08/08/2019	0.05	0.16	0.26	1.70	34.97
T4.8	08/08/2019	0.04	0.15	0.23	1.67	33.10
T4.12	08/08/2019	0.09	0.28	0.39	1.77	34.99
T4.16	21/07/2019	0.08	0.25	0.41	1.74	36.85
T0	26/08/2019	0.06	0.21	0.29	1.75	35.72
T0	26/08/2019	0.05	0.19	0.24	1.66	32.71
T0	26/08/2019	0.04	0.18	0.20	1.64	30.23
T1.2	26/08/2019	0.07	0.21	0.35	1.71	36.51
T1.4	26/08/2019	0.10	0.29	0.40	1.69	36.03
T1.8	26/08/2019	0.05	0.17	0.28	1.59	34.83
T1.12	26/08/2019	0.06	0.20	0.23	1.68	33.80
T1.16	26/08/2019	0.04	0.15	0.19	1.53	29.54
T2.2	26/08/2019	0.06	0.27	0.22	1.76	33.11
T2.4	26/08/2019	0.04	0.23	0.19	1.81	34.20
T2.8	26/08/2019	0.05	0.27	0.19	1.76	35.93
T2.12	26/08/2019	0.06	0.13	0.20	1.55	32.76
T2.16	26/08/2019	0.05	0.12	0.18	1.48	33.68
T3.2	26/08/2019	0.04	0.24	0.19	1.62	29.77
T3.4	26/08/2019	0.04	0.18	0.26	1.47	29.97
T3.8	26/08/2019	0.05	0.23	0.22	1.53	30.63
T3.12	26/08/2019	0.04	0.30	0.21	1.70	29.48
T3.16	26/08/2019	0.05	0.25	0.25	1.61	31.32
T4.2	26/08/2019	0.04	0.17	0.19	1.49	32.27
T4.4	26/08/2019	0.03	0.13	0.17	1.53	30.61
T4.8	26/08/2019	0.03	0.14	0.17	1.30	27.49
T4.12	26/08/2019	0.04	0.28	0.21	1.85	30.21
T4.16	26/08/2019	0.04	0.27	0.24	1.66	29.49
T0	25/09/2019	0.03	0.14	0.17	1.64	29.95
T0	25/09/2019	0.03	0.15	0.16	1.38	27.45
T0	25/09/2019	0.02	0.16	0.16	1.42	28.23
T1.2	25/09/2019	0.04	0.30	0.22	1.58	28.62
T1.4	25/09/2019	0.05	0.31	0.28	1.65	31.70
T1.8	25/09/2019	0.03	0.22	0.17	1.37	27.32
T1.12	25/09/2019	0.04	0.10	0.17	1.57	32.23
T1.16	25/09/2019	0.03	0.13	0.15	1.44	29.82
T4.2	25/09/2019	0.04	0.12	0.15	1.61	30.13
T4.4	25/09/2019	0.03	0.15	0.16	1.62	29.45
T4.8	25/09/2019	0.03	0.17	0.15	1.58	28.74
T4.12	25/09/2019	0.02	0.11	0.15	1.37	26.18
T4.6	25/09/2019	0.03	0.10	0.14	1.34	26.44
T0	18/10/2019	0.10	0.30	0.84	1.66	25.96

Proovi tähis	Proovivõtu-kuupäev	Nitrite [µmol N/l]	OrthoPhosphate [µmol P/l]	Nitrate Nitrite [µmol N/l]	Total P [µmol P/l]	Total N [µmol N/l]
T0	18/10/2019	0.09	0.30	0.79	1.49	24.29
T0	18/10/2019	0.09	0.32	0.81	1.62	26.63
T1.2	18/10/2019	0.04	0.14	0.18	1.47	25.85
T1.4	18/10/2019	0.04	0.17	0.16	1.51	28.87
T1.8	18/10/2019	0.03	0.15	0.16	1.39	25.30
T1.12	18/10/2019	0.03	0.15	0.15	1.47	27.34
T1.16	18/10/2019	0.02	0.14	0.15	1.27	23.37
T2.2	18/10/2019	0.04	0.17	0.15	1.38	23.22
T2.4	18/10/2019	0.04	0.15	0.15	1.75	25.88
T2.8	18/10/2019	0.03	0.17	0.17	1.18	21.05
T2.12	18/10/2019	0.10	0.13	0.54	1.11	47.61
T2.16	18/10/2019	0.03	0.17	0.16	1.51	24.27
T3.2	18/10/2019	0.02	0.16	0.15	1.59	24.57
T3.4	18/10/2019	0.04	0.13	0.15	1.64	27.58
T3.8	18/10/2019	0.03	0.21	0.15	1.50	24.62
T3.12	18/10/2019	0.02	0.15	0.14	1.06	19.74
T3.16	18/10/2019	0.02	0.15	0.14	1.29	22.99
T4.2	18/10/2019	0.02	0.18	0.16	1.56	26.81
T4.4	18/10/2019	0.03	0.20	0.16	1.61	26.84
T4.8	18/10/2019	0.03	0.17	0.16	1.45	25.51
T4.12	18/10/2019	0.03	0.14	0.15	1.45	26.25
T4.16	18/10/2019	0.02	0.16	0.16	1.22	21.74

Tabel 3. 2020. aasta esimese eksperimendi veekeemia analüüside tulemused.

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot}, µg N/l	P_{tot}, µg P/l	NO2, µg N/l
0	08/06/2020	5.30	10.83	245.7	34.42	0.59
0	08/06/2020	5.53	10.32	351.6	25.89	0.31
0	08/06/2020	3.60	9.15	346.6	23.99	0.31
1_3	08/06/2020	3.51	9.18	250.5	34.01	0.50
1_5	08/06/2020	3.13	9.38	263.1	28.27	0.22
1_9	08/06/2020	3.06	8.93	275.4	31.63	0.30
1_12	08/06/2020	3.13	8.68	210.0	34.61	0.25
1_15	08/06/2020	3.48	8.70	201.1	37.62	0.21
2_3	08/06/2020	3.50	8.32	206.7	36.21	0.37
2_5	08/06/2020	2.93	9.63	170.5	36.86	0.33
2_9	08/06/2020	2.78	8.49	155.8	35.06	0.33
2_12	08/06/2020	3.27	9.05	196.7	38.13	0.23
2_15	08/06/2020	3.23	7.71	150.8	31.55	0.22
3_3	08/06/2020	3.01	6.87	111.0	24.22	0.46
3_5	08/06/2020	2.71	7.68	164.9	29.60	0.46
3_9	08/06/2020	2.75	7.10	143.7	25.86	0.32
3_12	08/06/2020	3.20	7.05	129.7	28.82	0.25
3_15	08/06/2020	3.28	6.76	149.1	31.40	0.22
4_3	08/06/2020	3.05	8.03	105.2	24.76	0.19
4_5	08/06/2020	2.74	5.01	53.2	16.53	0.29
4_9	08/06/2020	2.76	6.50	115.7	24.25	0.35
4_12	08/06/2020	3.44	7.12	136.4	30.37	0.27
4_15	08/06/2020	3.13	6.99	146.8	31.39	0.18
0	11/06/2020	2.60	12.53	280.3	13.25	0.44
0	11/06/2020	2.60	11.82	304.6	13.40	0.50
0	11/06/2020	2.30	11.73	263.7	14.14	0.40
1_3	11/06/2020	2.83	9.13	313.8	12.76	0.47
1_5	11/06/2020	2.98	10.06	252.1	11.02	0.40
1_9	11/06/2020	2.60	10.07	270.4	11.98	0.38
1_12	11/06/2020	1.94	9.76	231.8	13.50	0.37
1_15	11/06/2020	1.93	9.24	238.2	9.62	0.32
2_3	11/06/2020	2.57	8.21	232.2	11.11	0.33
2_5	11/06/2020	2.71	9.30	220.9	10.63	0.30
2_9	11/06/2020	1.92	12.27	186.3	13.78	0.28
2_12	11/06/2020	1.98	9.83	237.5	17.00	0.30
2_15	11/06/2020	2.50	8.03	189.3	10.8	0.26
3_3	11/06/2020	2.52	8.53	215.0	12.3	0.30
3_5	11/06/2020	3.04	10.10	289.0	19.8	0.36
3_9	11/06/2020	2.30	7.84	217.7	14.6	0.28
3_12	11/06/2020	3.20	9.84	255.2	17.4	0.33
3_15	11/06/2020	1.89	9.29	226.4	12.9	0.24
4_3	11/06/2020	2.26	9.48	247.6	14.8	0.27
4_5	11/06/2020	1.92	7.22	115.6	5.4	0.19
4_9	11/06/2020	2.36	8.61	225.0	12.2	0.29
4_12	11/06/2020	1.87	8.24	212.6	8.9	0.29
4_15	11/06/2020	1.76	8.89	232.2	14.5	0.34
0	14/06/2020	1.80	9.06	122.9	10.5	0.25
0	14/06/2020	1.96	12.85	272.4	18.8	0.38

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot}, µg N/l	P_{tot}, µg P/l	NO2, µg N/l
0	14/06/2020	1.96	12.75	242.2	16.1	0.34
1_3	14/06/2020	2.21	11.43	287.6	17.3	0.46
1_5	14/06/2020	2.68	8.78	210.4	14.1	0.35
1_9	14/06/2020	2.81	9.06	195.8	9.7	0.38
1_12	14/06/2020	1.92	9.20	203.6	9.4	0.38
1_15	14/06/2020	1.88	8.24	196.5	9.3	0.30
2_3	14/06/2020	2.24	11.91	255.3	19.5	0.50
2_5	14/06/2020	2.12	10.89	263.5	18.7	0.40
2_9	14/06/2020	2.57	8.96	262.1	17.4	0.38
2_12	14/06/2020	2.00	8.23	182.3	19.8	0.37
2_15	14/06/2020	1.72	8.82	273.3	14.0	0.37
3_3	14/06/2020	2.05	11.12	270.1	16.6	0.44
3_5	14/06/2020	2.49	7.44	377.1	16.4	0.39
3_9	14/06/2020	2.74	7.52	289.2	14.6	0.34
3_12	14/06/2020	2.10	7.86	251.8	11.0	0.28
3_15	14/06/2020	1.89	7.76	240.7	15.6	0.28
4_3	14/06/2020	2.03	12.45	274.2	21.63	0.40
4_5	14/06/2020	1.94	12.06	258.8	16.58	0.40
4_9	14/06/2020	3.04	9.61	187.0	13.94	0.31
4_12	14/06/2020	1.98	9.30	227.7	10.31	0.33
4_15	14/06/2020	1.83	8.94	248.0	8.99	0.34
0	18/06/2020	1.74	11.37	256.7	12.21	0.33
0	18/06/2020	2.36	13.13	278.9	17.57	0.36
0	18/06/2020	2.50	12.10	246.6	13.69	0.38
1_3	18/06/2020	2.52	8.62	251.0	15.93	0.45
1_5	18/06/2020	3.03	9.65	256.5	12.65	0.57
1_9	18/06/2020	1.83	7.45	169.4	8.54	0.31
1_12	18/06/2020	2.26	9.78	305.7	13.22	0.39
1_15	18/06/2020	1.95	8.85	233.9	10.36	0.39
2_3	18/06/2020	1.95	8.95	253.2	11.25	0.42
2_5	18/06/2020	1.95	7.95	270.4	15.64	0.35
2_9	18/06/2020	2.05	8.77	235.2	16.57	0.41
2_12	18/06/2020	2.07	8.74	186.1	12.79	0.31
2_15	18/06/2020	2.04	10.28	279.3	15.17	0.44
3_3	18/06/2020	1.89	10.06	288.6	12.51	0.35
3_5	18/06/2020	1.96	6.72	225.4	4.34	0.31
3_9	18/06/2020	2.60	7.83	148.9	2.75	0.24
3_12	18/06/2020	2.16	9.02	253.2	9.60	0.32
3_15	18/06/2020	2.61	9.07	292.7	9.15	0.31
4_3	18/06/2020	2.29	11.19	249.9	8.71	0.38
4_5	18/06/2020	2.62	8.54	181.2	7.54	0.29
4_9	18/06/2020	3.11	8.89	245.9	11.55	0.31
4_12	18/06/2020	1.65	8.20	223.5	14.34	0.39
4_15	18/06/2020	1.97	9.07	260.8	5.16	0.30
0	22/06/2020	3.32	13.35	272.8	15.16	0.58
0	22/06/2020	2.58	13.57	226.0	13.68	0.49
0	22/06/2020	3.03	14.64	309.0	16.44	0.57
1_3	22/06/2020	2.10	9.36	206.0	8.73	0.43
1_9	22/06/2020	1.93	8.50	274.9	7.91	0.44
1_12	22/06/2020	2.01	8.36	303.5	10.94	0.48

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot}, µg N/l	P_{tot}, µg P/l	NO2, µg N/l
1_15	22/06/2020	2.16	8.69	262.3	8.46	0.49
2_3	22/06/2020	2.46	10.71	305.8	18.33	0.72
2_5	22/06/2020	2.49	9.10	333.2	19.46	0.68
2_9	22/06/2020	2.49	8.45	213.7	17.08	0.45
2_12	22/06/2020	2.01	8.21	241.3	11.86	0.45
2_15	22/06/2020	2.53	8.15	257.5	9.32	0.50
3_3	22/06/2020	2.54	10.85	304.3	18.12	0.57
3_5	22/06/2020	2.22	9.28	287.3	18.81	0.52
3_9	22/06/2020	2.93	8.56	294.1	11.68	0.54
3_12	22/06/2020	1.96	8.50	293.1	10.49	0.47
3_15	22/06/2020	2.17	6.91	247.7	4.50	0.46
4_3	22/06/2020	2.63	12.34	320.2	16.47	0.67
4_5	22/06/2020	2.40	9.62	265.8	14.88	0.62
4_9	22/06/2020	2.44	9.30	265.1	17.75	0.59
4_15	22/06/2020	2.04	8.08	190.6	7.44	0.51
0	25/06/2020	4.01	8.22	162.3	37.93	0.69
0	25/06/2020	5.10	8.59	175.3	29.31	0.73
0	25/06/2020	3.76	7.84	167.8	28.25	0.78
1_3	25/06/2020	2.71	5.91	121.7	21.60	0.52
1_5	25/06/2020	2.17	5.74	136.2	24.21	0.44
1_9	25/06/2020	2.75	5.26	169.9	21.49	0.39
1_12	25/06/2020	3.13	4.94	165.7	21.00	0.47
1_15	25/06/2020	3.04	4.96	219.1	20.16	0.32
2_3	25/06/2020	2.77	5.98	235.2	30.84	0.62
2_5	25/06/2020	2.27	5.51	251.9	23.65	0.45
2_9	25/06/2020	3.64	5.34	252.5	21.20	0.40
2_12	25/06/2020	2.61	4.09	192.4	15.95	0.35
2_15	25/06/2020	2.76	5.40	242.1	29.95	0.40
3_3	25/06/2020	2.74	7.22	195.3	31.30	0.52
3_5	25/06/2020	2.36	4.40	247.3	26.41	0.44
3_9	25/06/2020	2.41	5.48	205.5	24.98	0.43
3_12	25/06/2020	2.48	4.87	173.4	21.39	0.34
3_15	25/06/2020	3.03	4.52	158.9	18.59	0.26
4_3	25/06/2020	3.18	6.87	168.7	20.04	0.66
4_5	25/06/2020	2.44	7.03	144.0	25.94	0.54
4_9	25/06/2020	2.81	5.61	163.4	25.47	0.41
4_12	25/06/2020	2.62	4.89	173.7	21.42	0.52
4_15	25/06/2020	2.22	4.56	149.2	16.86	0.44
1.5	22/06/2020	1.91	9.90	218.4	21.90	0.39
4.12	22/06/2020	2.22	8.93	233.2	11.93	0.53

Tabel 4. 2020. aasta teise eksperimendi veekeemia analüüside tulemused.

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
0	23/07/2020	2.53	6.79	159.1	20.06	0.47
0	23/07/2020	3.23	6.12	110.9	16.09	0.33
0	23/07/2020	2.22	5.05	88.0	13.98	0.42
1_3	23/07/2020	3.30	5.85	214.2	18.52	0.32
1_5	23/07/2020	2.75	5.55	124.9	18.97	0.35
1_9	23/07/2020	2.38	4.73	143.9	15.14	0.32
1_12	23/07/2020	3.58	4.74	115.8	13.97	0.31
1_15	23/07/2020	2.76	4.75	162.3	14.93	0.27
2_3	23/07/2020	3.86	5.78	161.3	17.59	0.40
2_5	23/07/2020	3.22	5.85	112.1	17.88	0.34
2_9	23/07/2020	3.07	4.73	137.4	14.33	0.26
2_12	23/07/2020	3.09	4.63	133.3	13.16	0.28
2_15	23/07/2020	3.62	5.09	139.5	12.56	0.25
3_3	23/07/2020	3.63	5.08	112.7	15.48	0.50
3_5	23/07/2020	3.69	4.97	132.1	16.13	0.24
3_9	23/07/2020	2.20	4.30	86.2	11.74	0.29
3_12	23/07/2020	2.76	4.75	77.9	11.52	0.22
3_15	23/07/2020	2.04	4.14	103.2	12.83	0.36
4_3	23/07/2020	3.04	4.86	85.5	14.53	0.44
4_5	23/07/2020	4.48	5.75	173.4	15.54	0.42
4_9	23/07/2020	2.00	4.71	104.5	14.86	0.38
4_12	23/07/2020	2.45	5.29	174.5	18.57	0.27
4_15	23/07/2020	2.14	4.85	94.6	13.33	0.33
0	27/07/2020	3.56	11.24	171.7	29.39	0.46
0	27/07/2020	2.97	10.36	195.4	29.51	0.53
0	27/07/2020	3.00	10.65	199.6	30.60	0.59
1_3	27/07/2020	1.70	7.17	180.6	26.17	0.31
1_5	27/07/2020	2.42	5.29	176.8	23.32	0.40
1_9	27/07/2020	2.34	5.41	240.4	20.66	0.26
1_12	27/07/2020	2.43	5.05	184.7	23.62	0.24

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
1_15	27/07/2020	1.63	4.46	197.3	23.44	0.31
2_3	27/07/2020	2.24	6.11	194.5	26.45	0.39
2_5	27/07/2020	1.88	7.56	228.9	33.08	0.40
2_9	27/07/2020	1.76	5.87	192.1	25.70	0.32
2_12	27/07/2020	2.26	5.57	177.5	24.56	0.30
2_15	27/07/2020	2.77	5.58	164.9	25.93	0.31
3_3	27/07/2020	2.51	7.29	197.8	28.88	0.41
3_5	27/07/2020	1.92	7.17	157.6	33.53	0.33
3_9	27/07/2020	1.83	5.47	164.9	25.90	0.35
3_12	27/07/2020	2.52	5.71	170.5	26.24	0.31
3_15	27/07/2020	2.26	5.31	189.0	24.44	0.33
4_3	27/07/2020	1.92	7.39	216.6	29.32	0.40
4_5	27/07/2020	2.40	7.28	187.8	30.50	0.38
4_9	27/07/2020	1.92	6.43	190.9	27.11	0.34
4_12	27/07/2020	2.23	5.91	182.6	27.11	0.36
4_15	27/07/2020	1.86	5.71	228.4	24.95	0.37
0	30/07/2020	2.71	8.13	201.6	31.10	0.21
0	30/07/2020	2.49	8.18	210.2	34.04	0.20
0	30/07/2020	2.43	7.02	187.6	27.02	0.19
1_3	30/07/2020	2.68	4.95	200.6	27.27	0.15
1_5	30/07/2020	3.58	4.30	185.1	25.89	0.19
1_9	30/07/2020	2.08	2.12	178.3	23.49	0.20
1_12	30/07/2020	2.08	2.02	181.3	26.61	0.23
1_15	30/07/2020	2.20	2.53	211.6	24.54	0.24
2_3	30/07/2020	2.10	4.97	171.8	25.97	0.21
2_5	30/07/2020	2.03	2.33	194.3	27.33	0.16
2_9	30/07/2020	2.11	3.55	160.3	24.79	0.09
2_12	30/07/2020	2.01	4.60	180.2	26.02	0.11
2_15	30/07/2020	2.10	2.44	200.8	26.95	0.12
3_3	30/07/2020	2.06	6.51	224.1	29.18	0.17
3_5	30/07/2020	2.01	3.78	186.6	25.56	0.20

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
3_9	30/07/2020	1.97	2.72	194.0	21.68	0.20
3_12	30/07/2020	1.85	2.43	192.9	21.21	0.13
3_15	30/07/2020	1.91	1.77	192.5	24.97	0.21
4_3	30/07/2020	1.91	4.44	173.2	23.79	0.30
4_5	30/07/2020	1.81	2.95	186.9	22.28	0.01
4_9	30/07/2020	1.69	4.46	175.0	25.07	0.00
4_12	30/07/2020	1.78	2.32	191.5	24.63	0.05
4_15	30/07/2020	1.77	1.96	177.5	24.92	0.12
0	03/08/2020	4.27	17.16	231.5	49.20	0.60
0	03/08/2020	3.80	14.28	216.5	44.34	0.49
0	03/08/2020	4.10	20.10	232.7	57.34	0.60
1_3	03/08/2020	2.83	11.01	224.0	40.60	0.42
1_5	03/08/2020	1.93	8.84	183.7	32.26	0.37
1_9	03/08/2020	2.00	8.04	252.2	38.92	0.41
1_12	03/08/2020	2.04	5.81	242.5	36.77	0.45
1_15	03/08/2020	2.09	4.89	241.8	32.50	0.40
2_3	03/08/2020	2.68	9.53	246.6	42.10	0.74
2_5	03/08/2020	2.06	11.07	237.7	42.71	0.56
2_9	03/08/2020	1.90	7.39	253.6	41.53	0.49
2_12	03/08/2020	1.88	7.29	221.3	33.96	0.37
2_15	03/08/2020	1.95	5.47	275.2	38.21	0.36
3_3	03/08/2020	2.55	14.72	324.0	42.00	0.51
3_5	03/08/2020	2.07	10.21	294.3	44.11	0.33
3_9	03/08/2020	1.96	9.51	317.2	43.02	0.29
3_12	03/08/2020	1.94	6.08	221.3	42.99	0.34
3_15	03/08/2020	1.95	5.83	177.7	34.60	0.26
4_3	03/08/2020	2.12	9.53	156.8	36.52	0.49
4_5	03/08/2020	2.24	10.73	211.4	46.87	0.52
4_9	03/08/2020	1.98	10.18	218.3	43.58	0.34
4_12	03/08/2020	1.87	7.96	187.5	38.11	0.27
4_15	03/08/2020	1.75	3.88	136.3	28.22	0.27

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
0	06/08/2020	1.79	19.23	179.7	45.85	0.32
0	06/08/2020	1.65	19.69	193.9	48.61	0.39
0	06/08/2020	1.57	16.98	175.7	42.36	0.36
1_3	06/08/2020	1.54	8.75	153.6	33.85	0.26
1_5	06/08/2020	1.55	12.31	227.0	45.61	0.31
1_9	06/08/2020	1.80	7.91	201.5	37.93	0.30
1_12	06/08/2020	1.95	9.66	187.8	43.90	0.38
1_15	06/08/2020	1.71	6.93	134.3	32.59	0.24
2_3	06/08/2020	1.73	10.42	160.6	41.89	0.40
2_5	06/08/2020	1.67	8.20	175.0	36.66	0.38
2_9	06/08/2020	1.82	9.74	206.2	43.32	0.37
2_12	06/08/2020	1.71	6.40	142.9	31.80	0.32
2_15	06/08/2020	1.71	8.84	166.1	37.73	0.33
3_3	06/08/2020	1.77	13.29	173.7	48.66	0.31
3_5	06/08/2020	1.69	11.89	125.1	36.11	0.23
3_9	06/08/2020	1.71	6.68	122.4	27.21	0.23
3_12	06/08/2020	1.65	5.13	133.7	32.31	0.24
3_15	06/08/2020	1.72	7.03	170.4	32.75	0.33
4_3	06/08/2020	1.56	17.25	244.8	53.64	0.50
4_5	06/08/2020	1.72	14.24	209.1	42.94	0.36
4_9	06/08/2020	1.65	9.72	189.9	46.48	0.41
4_12	06/08/2020	1.61	5.73	157.2	30.63	0.25
4_15	06/08/2020	1.53	5.70	143.1	27.33	0.24
0	10/08/2020	1.74	9.57	190.3	36.25	0.57
0	10/08/2020	1.91	11.12	184.1	37.09	0.35
0	10/08/2020	1.79	14.35	197.5	49.52	0.61
1_3	10/08/2020	1.57	9.44	165.4	37.30	0.43
1_5	10/08/2020	1.57	6.49	188.0	37.83	0.40
1_9	10/08/2020	1.63	5.84	185.0	38.48	0.50
1_12	10/08/2020	1.54	7.20	201.6	44.82	0.52
1_15	10/08/2020	1.58	3.45	95.7	27.08	0.32

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
2_3	10/08/2020	1.62	9.28	207.0	39.85	0.57
2_5	10/08/2020	1.81	7.96	254.8	43.51	0.53
2_9	10/08/2020	1.58	6.19	258.0	35.72	0.51
2_12	10/08/2020	1.62	5.44	201.3	33.37	0.43
2_15	10/08/2020	1.53	3.84	226.2	36.44	0.43
3_3	10/08/2020	1.66	6.78	250.5	37.62	0.46
3_5	10/08/2020	1.63	7.51	259.2	40.38	0.41
3_9	10/08/2020	1.74	6.98	267.9	41.46	0.40
3_12	10/08/2020	1.74	7.19	247.5	36.67	0.33
3_15	10/08/2020	1.80	5.13	273.6	33.44	0.39
4_3	10/08/2020	1.89	6.74	282.3	39.36	0.43
4_5	10/08/2020	1.75	4.85	273.6	36.26	0.35
4_9	10/08/2020	1.66	5.60	332.4	36.53	0.42
4_12	10/08/2020	1.65	5.06	238.1	33.08	0.43
4_15	10/08/2020	1.64	8.16	214.5	36.75	0.45

Tabel 5. 2020. aasta kolmanda eksperimendi veekeemia analüüside tulemused.

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
0	04/09/2020	29.55	29.99	559.7	65.42	4.88
0	04/09/2020	31.80	32.81	593.8	75.53	4.50
0	04/09/2020	28.86	34.49	648.8	80.45	4.39
1_3	04/09/2020	34.98	24.98	541.9	68.83	4.66
1_5	04/09/2020	35.02	22.84	418.2	50.39	4.31
1_9	04/09/2020	32.52	20.27	476.7	44.89	4.19
1_12	04/09/2020	35.47	22.18	456.8	64.66	4.49
1_15	04/09/2020	35.26	21.24	569.7	51.38	3.57
2_3	04/09/2020	32.86	26.08	514.7	76.63	4.83
2_5	04/09/2020	33.97	20.15	462.1	58.26	4.48
2_9	04/09/2020	34.74	21.08	526.8	52.16	4.04
2_12	04/09/2020	36.29	19.99	560.4	57.48	4.50
2_15	04/09/2020	37.55	22.13	555.0	60.52	3.89
3_3	04/09/2020	35.20	26.12	542.5	66.92	4.19
3_5	04/09/2020	31.26	18.67	506.7	56.55	3.82
3_9	04/09/2020	31.46	17.04	479.7	55.64	3.64
3_12	04/09/2020	31.80	14.03	503.8	48.75	4.12
3_15	04/09/2020	34.72	16.11	527.5	51.85	4.49
4_3	04/09/2020	33.51	20.02	571.0	52.77	4.49
4_5	04/09/2020	29.44	14.66	514.8	57.95	4.18
4_9	04/09/2020	29.92	18.81	525.9	51.59	3.79
4_12	04/09/2020	31.40	18.45	593.0	45.74	3.92
4_15	04/09/2020	31.42	17.48	554.0	64.01	3.57
0	07/09/2020	25.25	23.15	385.2	46.62	2.21
0	07/09/2020	33.71	23.60	429.5	45.14	2.68
0	07/09/2020	29.77	25.25	438.5	51.14	2.43
1_3	07/09/2020	5.61	10.13	364.4	35.40	1.32
1_5	07/09/2020	3.22	20.03	402.0	48.36	0.57
1_9	07/09/2020	3.04	8.63	339.2	31.91	0.35
1_12	07/09/2020	2.88	8.05	323.6	31.26	0.27
1_15	07/09/2020	2.56	7.34	325.2	30.68	0.29
2_3	07/09/2020	13.45	10.53	358.6	39.74	7.37
2_5	07/09/2020	3.74	8.72	372.3	36.15	2.07
2_9	07/09/2020	2.37	8.20	318.9	31.48	0.27
2_12	07/09/2020	2.64	7.35	294.5	28.46	0.20
2_15	07/09/2020	2.22	7.04	325.5	29.58	0.14
3_3	07/09/2020	3.97	8.15	336.3	34.82	0.96
3_5	07/09/2020	3.06	6.67	330.5	31.56	0.26
3_9	07/09/2020	2.11	5.33	275.8	27.57	0.13
3_12	07/09/2020	2.32	5.09	246.9	24.01	0.14
3_15	07/09/2020	2.25	5.64	281.4	24.50	0.16
4_3	07/09/2020	7.90	10.22	343.8	36.46	1.61
4_5	07/09/2020	2.38	7.76	335.7	31.49	0.28
4_9	07/09/2020	2.26	5.48	302.5	26.15	0.15
4_12	07/09/2020	2.16	3.97	252.5	21.79	0.18
4_15	07/09/2020	2.24	3.84	247.6	20.23	0.19
0	11/09/2020	17.00	8.54	430.4	35.23	1.29

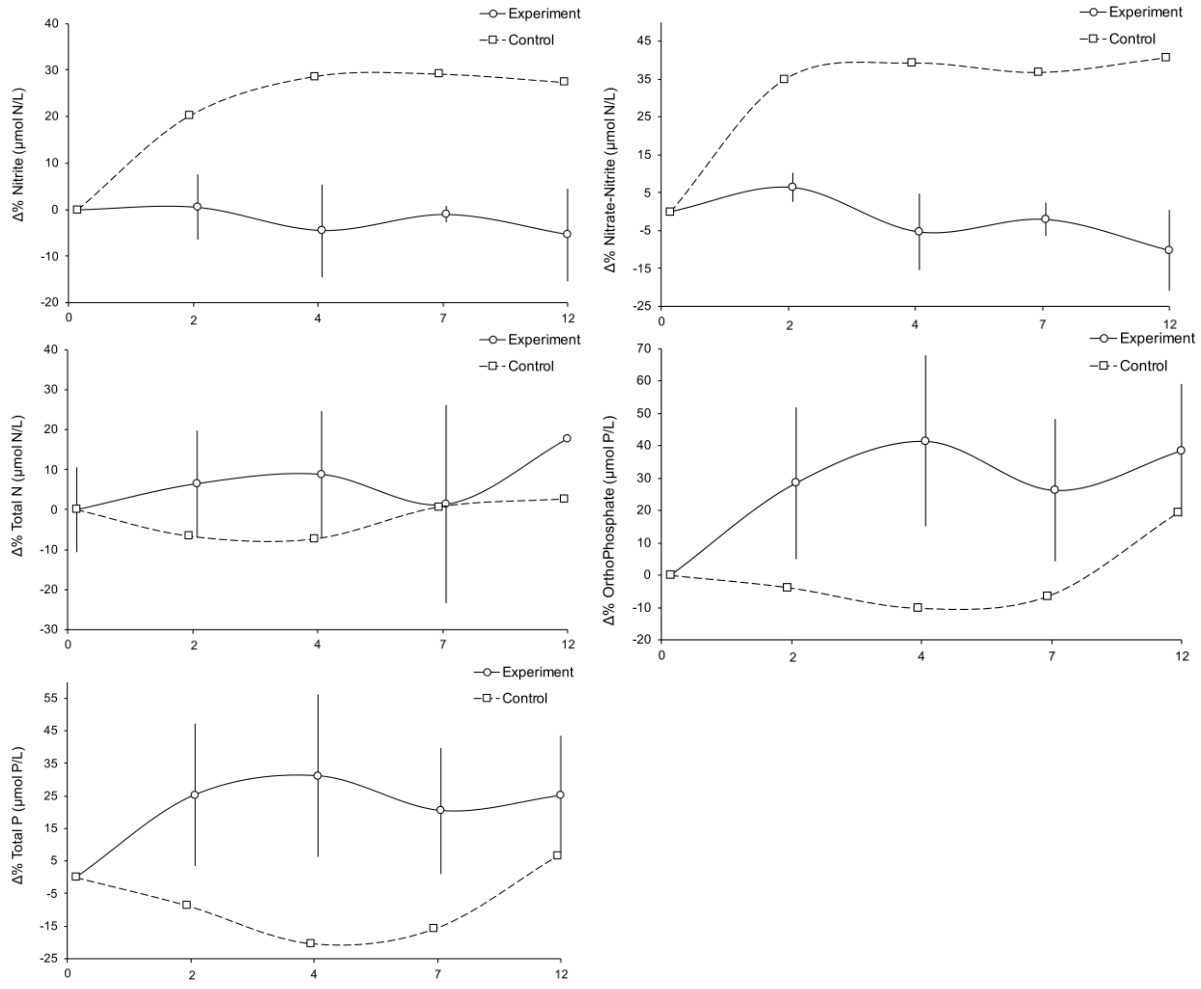
Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
0	11/09/2020	17.93	10.15	344.9	28.81	1.43
0	11/09/2020	17.17	7.61	373.3	34.39	1.39
1_3	11/09/2020	11.10	6.29	407.2	25.23	1.13
1_5	11/09/2020	8.44	6.32	386.5	23.31	0.88
1_9	11/09/2020	5.87	4.53	330.1	22.44	0.78
1_12	11/09/2020	4.92	3.53	332.1	21.54	0.67
1_15	11/09/2020	4.05	3.30	333.5	23.63	0.96
2_3	11/09/2020	11.64	7.03	386.8	26.69	1.46
2_5	11/09/2020	9.50	5.33	235.7	22.12	1.09
2_9	11/09/2020	6.63	4.10	223.5	20.25	0.78
2_12	11/09/2020	5.84	3.96	219.6	21.41	0.74
2_15	11/09/2020	5.09	3.52	304.1	22.30	0.69
3_3	11/09/2020	11.42	4.71	228.6	22.24	1.15
3_5	11/09/2020	7.99	3.59	233.3	23.34	0.97
3_9	11/09/2020	5.11	2.33	222.1	17.70	0.35
3_12	11/09/2020	4.69	2.12	190.0	17.69	0.62
3_15	11/09/2020	4.10	2.13	190.2	23.31	0.54
4_3	11/09/2020	12.34	6.23	224.4	20.66	1.20
4_5	11/09/2020	10.04	4.62	217.5	17.10	1.08
4_9	11/09/2020	7.02	3.20	173.8	15.92	0.57
4_12	11/09/2020	5.61	2.65	233.9	17.92	0.72
4_15	11/09/2020	4.53	2.40	226.5	19.86	0.62
0	14/09/2020	21.37	5.39	309.9	26.76	1.90
0	14/09/2020	23.12	6.98	264.6	25.78	2.10
0	14/09/2020	23.75	7.16	225.1	22.14	1.72
1_3	14/09/2020	5.71	27.28	357.9	91.00	1.00
1_5	14/09/2020	8.43	2.96	226.7	19.28	1.28
1_9	14/09/2020	5.18	1.87	233.1	19.45	0.93
1_12	14/09/2020	2.41	2.26	218.2	18.11	0.59
1_15	14/09/2020	1.53	1.44	229.6	22.01	0.38
2_3	14/09/2020	16.52	4.17	258.8	24.02	1.73
2_5	14/09/2020	9.72	2.48	237.8	19.16	1.30
2_9	14/09/2020	5.06	2.02	242.6	20.06	0.85
2_12	14/09/2020	2.64	1.68	238.7	18.78	0.64
2_15	14/09/2020	1.44	0.65	255.8	19.22	0.50
3_3	14/09/2020	15.69	3.18	291.1	20.57	1.75
3_5	14/09/2020	6.61	3.48	246.6	21.07	0.99
3_9	14/09/2020	2.97	1.94	228.8	17.45	0.70
3_12	14/09/2020	2.18	0.89	229.9	18.32	0.72
3_15	14/09/2020	1.16	0.66	231.7	17.56	0.27
4_3	14/09/2020	17.53	4.10	248.0	18.09	1.77
4_5	14/09/2020	13.53	2.59	239.1	20.27	1.45
4_9	14/09/2020	5.27	1.54	241.0	17.35	1.08
4_12	14/09/2020	2.06	0.66	198.4	14.59	0.54
4_15	14/09/2020	1.11	1.25	220.5	15.05	0.43
0	17/09/2020	10.64	5.15	198.4	21.02	1.69
0	17/09/2020	10.43	4.89	254.1	23.39	1.61
0	17/09/2020	10.89	5.55	233.3	25.00	1.76
1_3	17/09/2020	7.17	2.14	251.2	17.16	1.23

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
1_5	17/09/2020	3.11	1.43	214.1	16.97	0.72
1_9	17/09/2020	2.28	1.96	242.5	19.11	0.60
1_12	17/09/2020	1.84	0.95	240.0	15.17	0.47
1_15	17/09/2020	1.33	1.16	251.7	17.13	0.37
2_3	17/09/2020	7.10	4.15	271.5	21.38	1.15
2_5	17/09/2020	4.00	2.46	231.7	18.32	0.78
2_9	17/09/2020	2.30	2.33	237.3	19.56	0.72
2_12	17/09/2020	1.72	1.55	237.3	16.65	0.44
2_15	17/09/2020	1.41	1.08	271.8	16.30	0.37
3_3	17/09/2020	6.56	3.44	206.7	23.56	1.12
3_5	17/09/2020	3.10	2.69	180.9	17.58	0.57
3_9	17/09/2020	1.82	0.89	198.9	21.18	0.82
3_12	17/09/2020	1.40	0.75	201.4	23.50	0.54
3_15	17/09/2020	1.27	0.47	172.9	17.57	0.49
4_3	17/09/2020	6.78	2.26	204.8	22.25	1.49
4_5	17/09/2020	5.37	2.26	188.9	23.39	1.37
4_9	17/09/2020	2.96	0.93	176.5	21.20	0.78
4_12	17/09/2020	2.03	1.09	208.9	20.55	0.58
4_15	17/09/2020	1.46	0.67	166.0	21.40	0.55
0	21/09/2020	9.73	12.79	238.2	38.79	1.12
0	21/09/2020	8.88	10.42	257.1	34.17	1.31
0	21/09/2020	6.98	11.05	196.3	41.24	0.97
1_3	21/09/2020	5.67	6.54	340.0	41.89	1.42
1_5	21/09/2020	1.61	4.15	307.8	51.36	0.61
1_9	21/09/2020	1.23	3.28	229.3	34.38	0.44
1_12	21/09/2020	1.22	2.85	219.5	33.17	0.32
1_15	21/09/2020	1.25	2.53	297.6	36.84	0.35
2_3	21/09/2020	6.84	8.12	324.6	38.92	1.46
2_5	21/09/2020	3.80	5.36	309.1	32.61	0.94
2_9	21/09/2020	1.16	2.29	195.2	27.91	0.29
2_12	21/09/2020	1.15	2.14	191.9	34.36	0.16
2_15	21/09/2020	1.13	2.81	146.1	30.44	0.22
3_3	21/09/2020	1.59	4.48	134.9	33.62	0.61
3_5	21/09/2020	1.05	3.63	143.4	28.62	0.18
3_9	21/09/2020	1.08	3.72	172.1	32.15	0.18
3_12	21/09/2020	2.20	3.65	170.0	28.59	0.29
3_15	21/09/2020	1.00	2.59	120.2	25.01	0.19
4_3	21/09/2020	4.17	4.09	175.7	32.11	0.97
4_5	21/09/2020	2.02	3.37	129.7	24.91	0.68
4_9	21/09/2020	0.88	2.85	180.3	28.60	0.33
4_12	21/09/2020	0.82	2.69	141.3	25.40	0.33
4_15	21/09/2020	1.05	1.64	141.4	26.87	0.35
0	24/09/2020	19.04	25.01	343.1	44.65	2.03
0	24/09/2020	21.43	28.76	363.9	46.14	2.75
0	24/09/2020	16.94	23.91	302.0	38.44	1.52
1_3	24/09/2020	16.05	19.63	312.3	34.56	2.03
1_5	24/09/2020	11.34	15.81	273.6	30.20	0.84
1_9	24/09/2020	8.71	9.62	221.2	19.95	0.98
1_12	24/09/2020	7.59	10.05	228.7	22.72	0.89

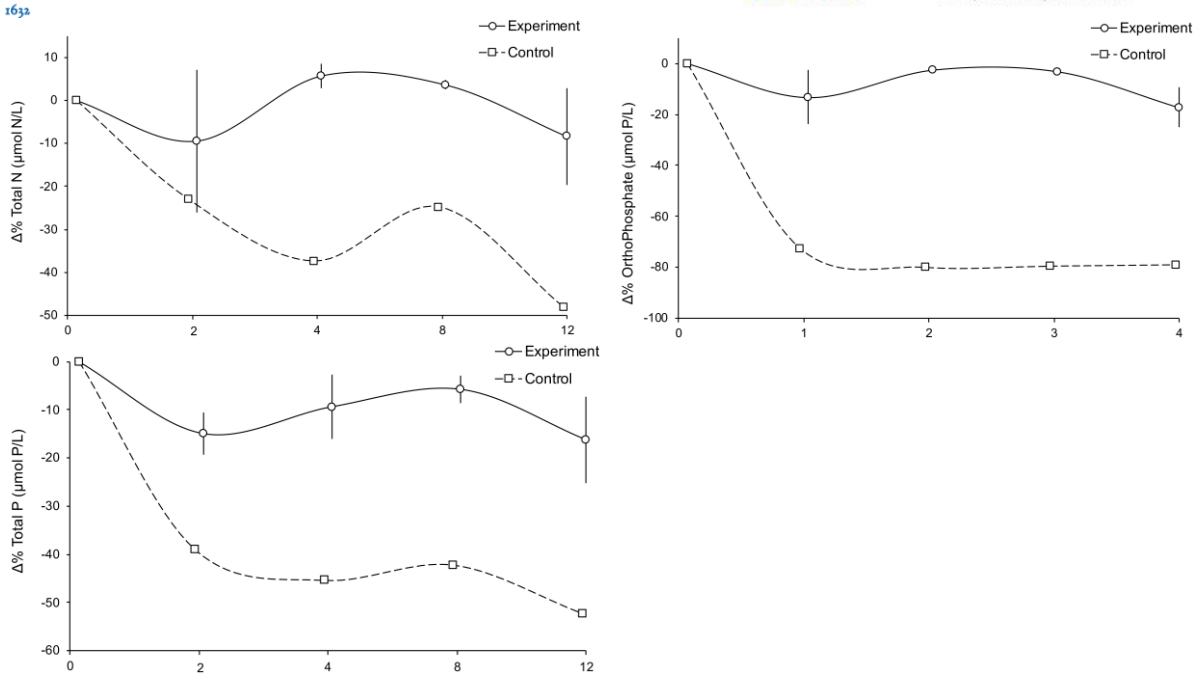
Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
1_15	24/09/2020	5.96	8.09	242.6	20.94	0.79
2_3	24/09/2020	14.45	17.24	276.1	30.68	1.19
2_5	24/09/2020	8.75	10.91	189.3	22.53	0.92
2_9	24/09/2020	8.07	8.91	169.3	18.79	0.83
2_12	24/09/2020	8.86	9.98	252.9	20.78	1.09
2_15	24/09/2020	6.57	8.25	244.6	19.15	0.84
3_3	24/09/2020	19.15	21.72	361.5	38.12	2.77
3_5	24/09/2020	10.42	14.11	263.0	28.55	1.36
3_9	24/09/2020	7.58	8.27	219.4	20.01	0.61
3_12	24/09/2020	7.48	11.08	286.9	24.38	1.09
3_15	24/09/2020	5.01	8.31	253.5	19.40	0.66
4_3	24/09/2020	13.85	19.26	276.9	34.14	1.53
4_5	24/09/2020	16.20	18.01	301.6	31.65	1.84
4_9	24/09/2020	10.14	10.39	224.1	21.41	1.26
4_12	24/09/2020	9.07	8.95	254.0	20.58	1.12
4_15	24/09/2020	6.29	7.05	225.5	20.64	0.82
0	28/09/2020	10.91	28.16	247.9	70.33	2.42
0	28/09/2020	10.41	25.74	223.8	60.25	2.39
0	28/09/2020	9.45	25.29	236.4	61.88	2.52
1_3	28/09/2020	5.84	13.69	245.3	53.37	1.91
1_5	28/09/2020	4.22	9.21	187.6	46.12	1.29
1_9	28/09/2020	1.77	11.56	183.7	38.76	0.69
1_12	28/09/2020	1.57	9.34	213.1	37.61	0.57
1_15	28/09/2020	1.24	7.10	183.1	37.43	0.53
2_3	28/09/2020	4.98	13.21	185.5	54.99	1.31
2_5	28/09/2020	3.76	10.82	204.7	40.01	1.27
2_9	28/09/2020	1.33	10.19	177.1	48.59	0.82
2_12	28/09/2020	1.41	9.56	176.4	40.65	0.71
2_15	28/09/2020	1.32	6.10	257.1	37.03	0.67
3_3	28/09/2020	5.28	13.53	260.9	53.89	1.54
3_5	28/09/2020	3.08	9.66	219.2	48.87	1.11
3_9	28/09/2020	1.65	7.10	198.8	41.58	0.60
3_12	28/09/2020	1.39	5.60	202.0	42.96	0.50
3_15	28/09/2020	1.12	5.96	179.2	35.19	0.33
4_3	28/09/2020	6.04	13.40	220.2	51.77	1.82
4_5	28/09/2020	3.88	13.08	230.3	48.18	1.26
4_9	28/09/2020	2.69	8.40	207.8	46.97	0.80
4_12	28/09/2020	1.63	7.34	212.5	37.01	0.61
4_15	28/09/2020	1.36	5.39	219.6	31.12	0.56
0	01/10/2020	6.23	6.75	196.7	24.13	0.90
0	01/10/2020	6.99	7.60	237.4	20.83	0.80
0	01/10/2020	6.81	7.49	197.5	34.77	0.80
1_3	01/10/2020	2.71	5.56	225.1	29.94	0.73
1_5	01/10/2020	2.27	6.46	294.5	30.35	0.64
1_9	01/10/2020	0.97	5.18	250.6	28.60	0.53
1_12	01/10/2020	0.81	4.49	240.9	24.87	0.55
1_15	01/10/2020	0.71	3.25	186.6	28.57	0.42
2_3	01/10/2020	3.39	7.17	212.5	34.41	0.58
2_5	01/10/2020	2.42	4.75	208.4	31.74	0.66

Proov	Proovivõtu-kuupäev	NO_X, µg N/l	PO_4, µg P/l	N_{tot} (UV), µg N/l	P_{tot} (UV), µg P/l	NO2, µg N/l
2_9	01/10/2020	1.01	3.50	223.3	30.45	0.54
2_12	01/10/2020	0.90	4.48	225.5	30.57	0.53
2_15	01/10/2020	0.80	2.71	230.3	25.42	0.31
3_3	01/10/2020	3.10	5.41	251.8	33.45	0.54
3_5	01/10/2020	2.17	3.09	203.1	30.55	0.47
3_9	01/10/2020	1.19	2.81	210.7	23.45	0.33
3_12	01/10/2020	1.01	3.24	226.0	27.04	0.29
3_15	01/10/2020	1.12	2.31	243.4	22.48	0.31
4_3	01/10/2020	3.23	3.68	240.8	28.88	0.50
4_5	01/10/2020	2.61	3.54	185.7	28.85	0.45
4_9	01/10/2020	0.92	4.21	205.6	28.85	0.40
4_12	01/10/2020	0.84	2.80	216.2	24.20	0.17
4_15	01/10/2020	1.40	2.78	234.9	30.78	0.29

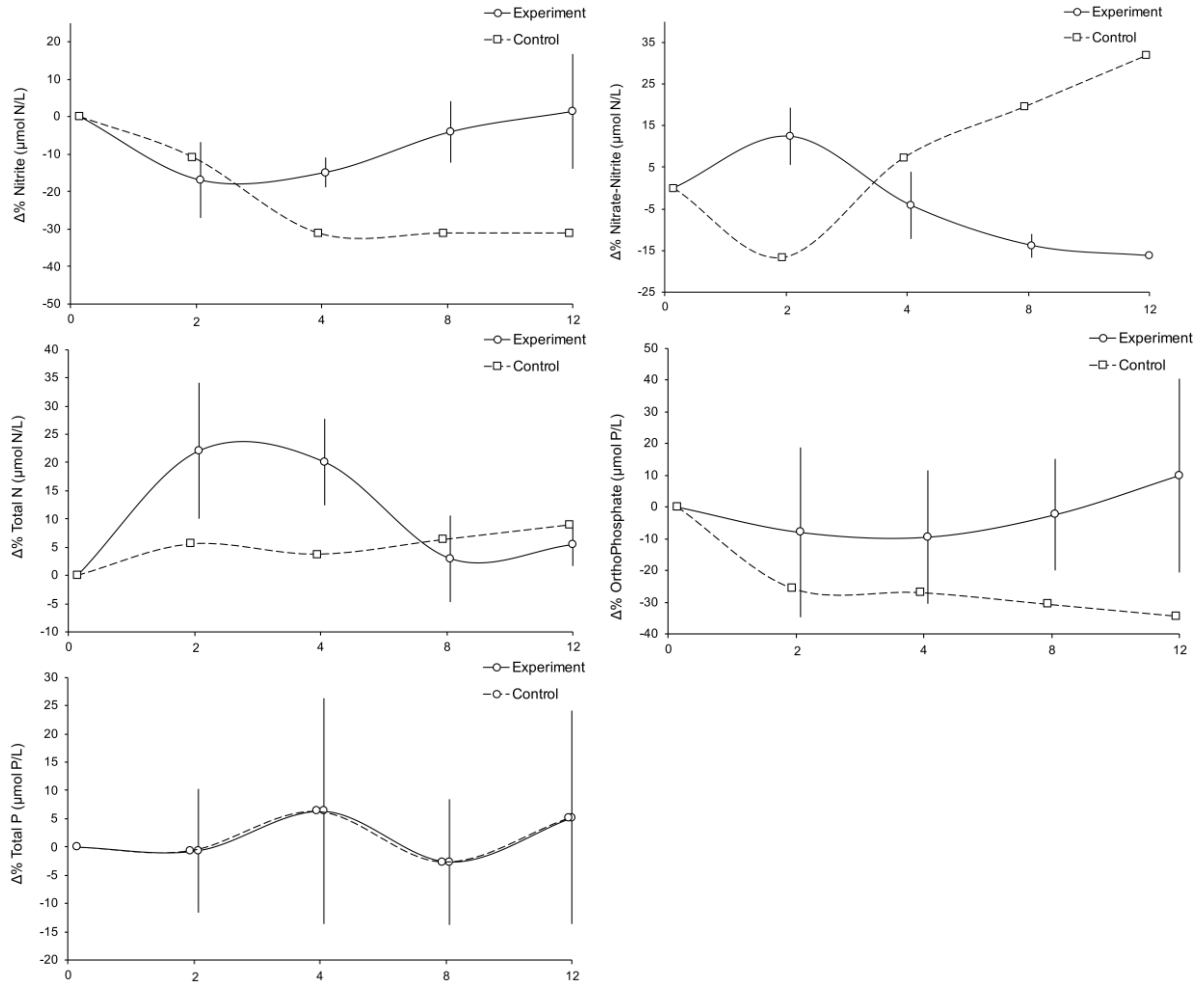
LISA 2



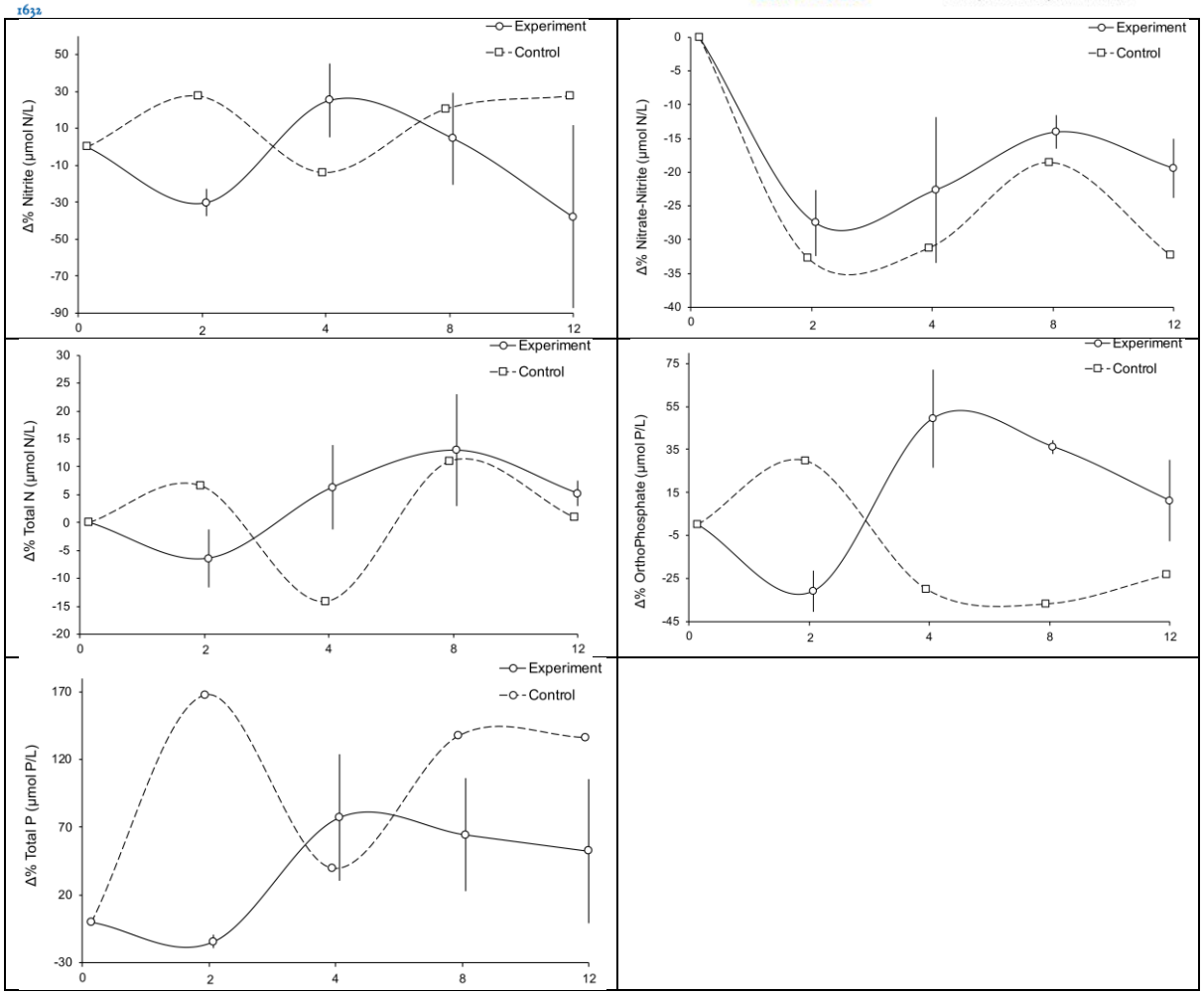
Joonis 1. Toitainete kontsentratsiooni muutus biofiltersüsteemis 25.04.2019 mõõtmistel. X – teljel märgitud mahutite rea number.



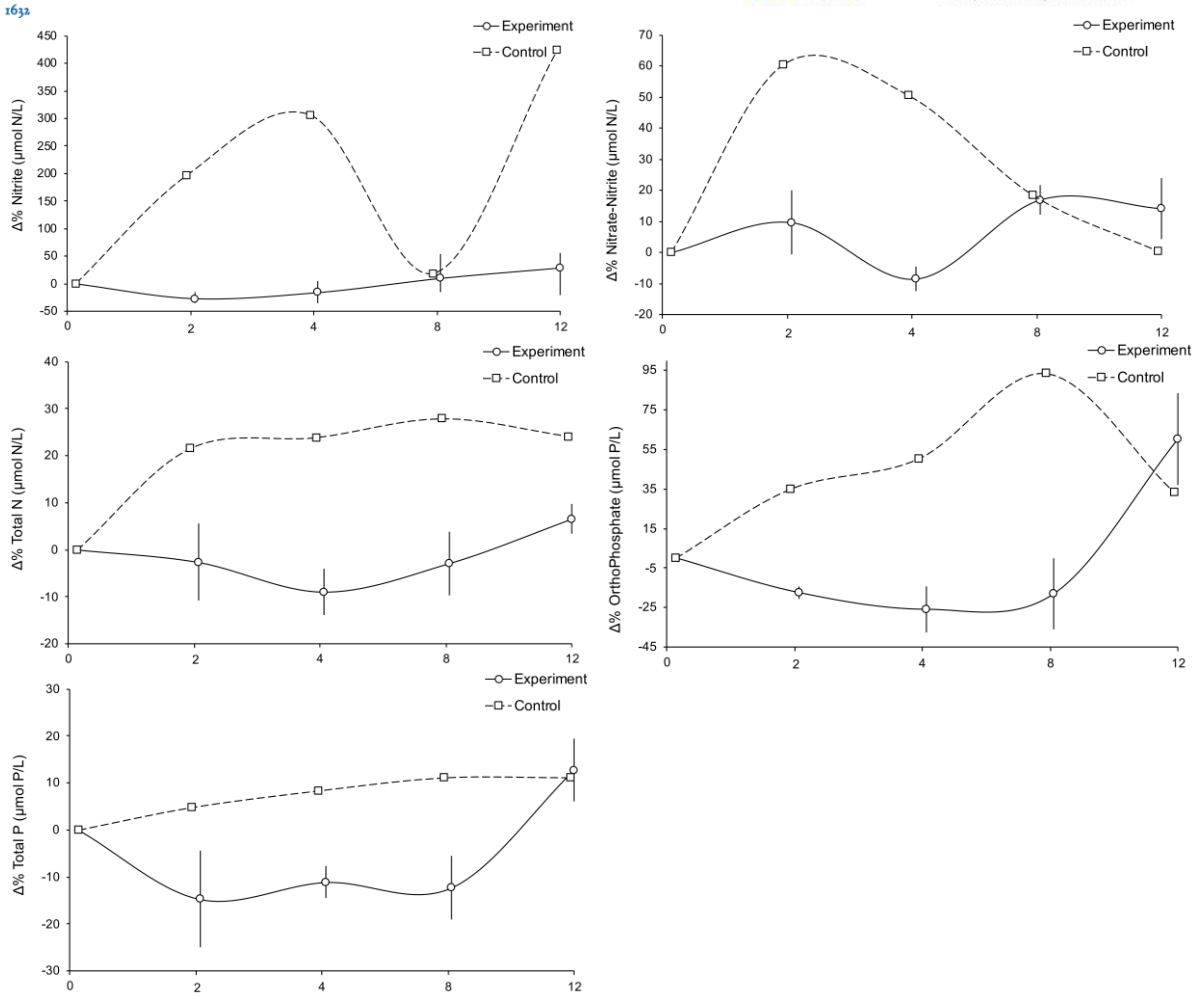
Joonis 2. Toitainete kontsentratsiooni muutus biofiltersüsteemis 02.05.2019 mõõtmistel. X – teljel märgitud mahutite rea number.



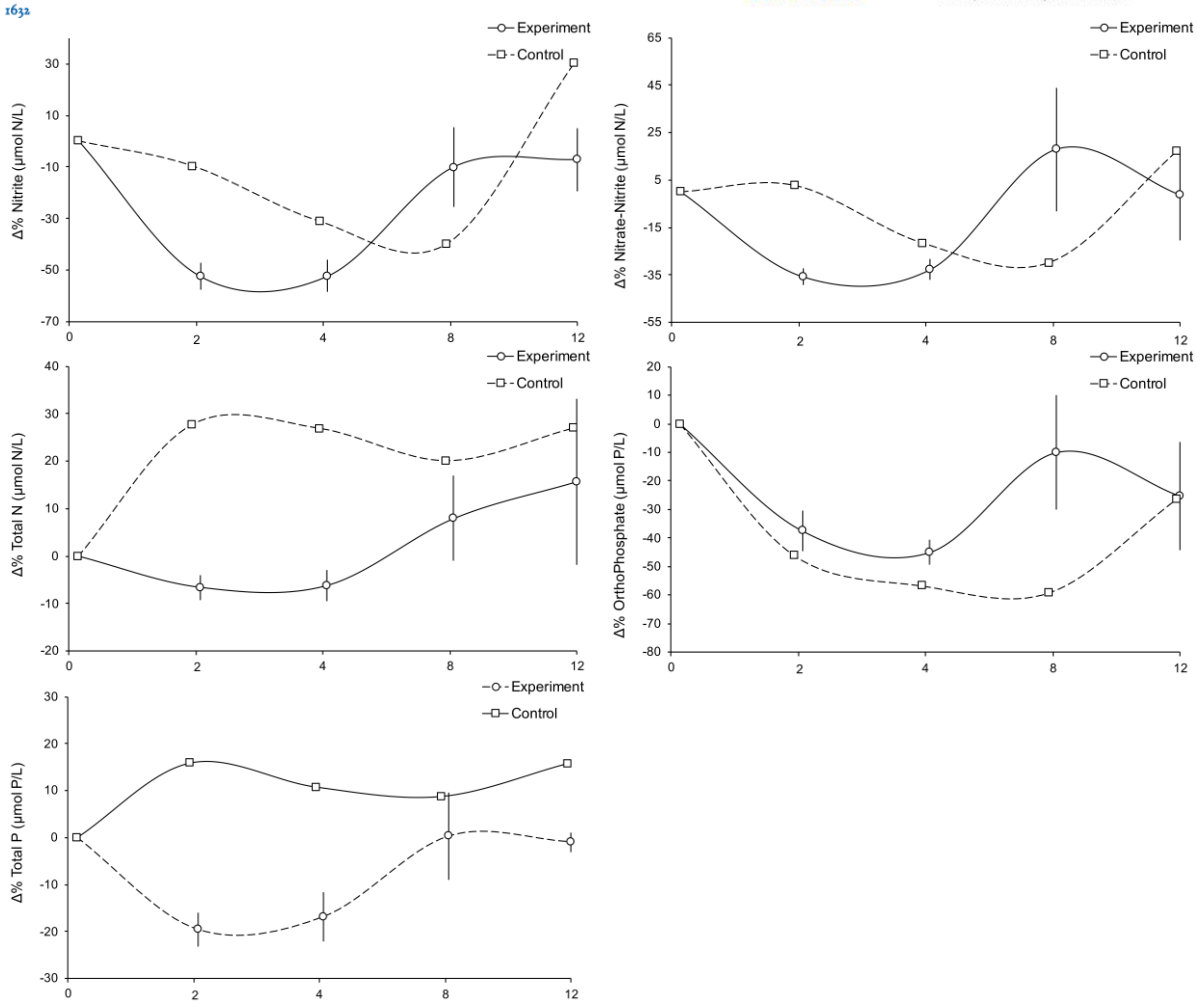
Joonis 3. Toitainete kontsentratsiooni muutus biofiltersüsteemis 04.06.2019 mõõtmistel. X – teljel märgitud mahutite rea number.



Joonis 4. Toitainete kontsentratsiooni muutus biofiltersüsteemis 18.06.2019 mõõtmistel. X – teljel märgitud mahutite rea number.



Joonis 5. Toitainete kontsentratsiooni muutus biofiltersüsteemis 30.07.2019 mõõtmistel. X – teljel märgitud mahutite rea number.



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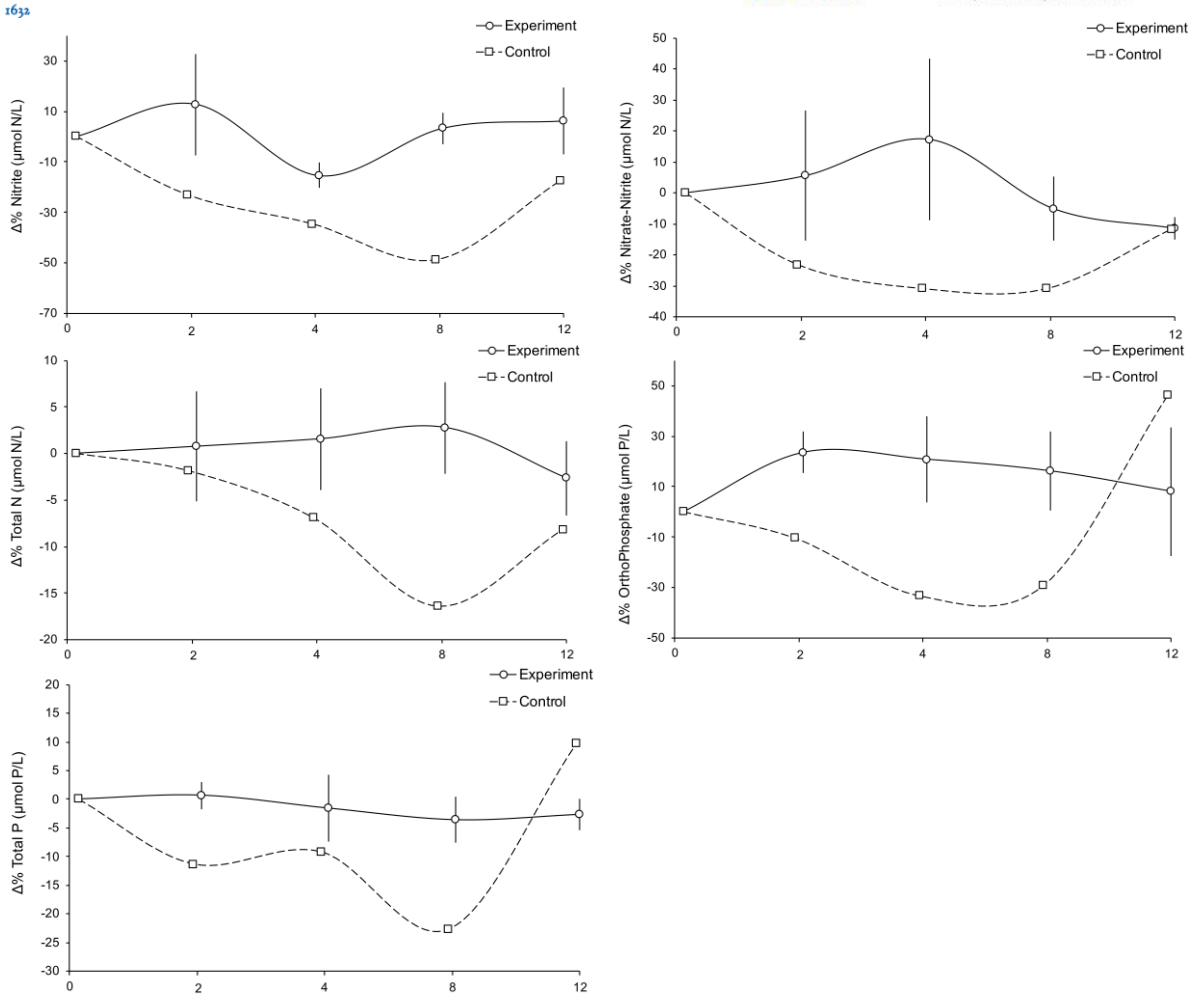

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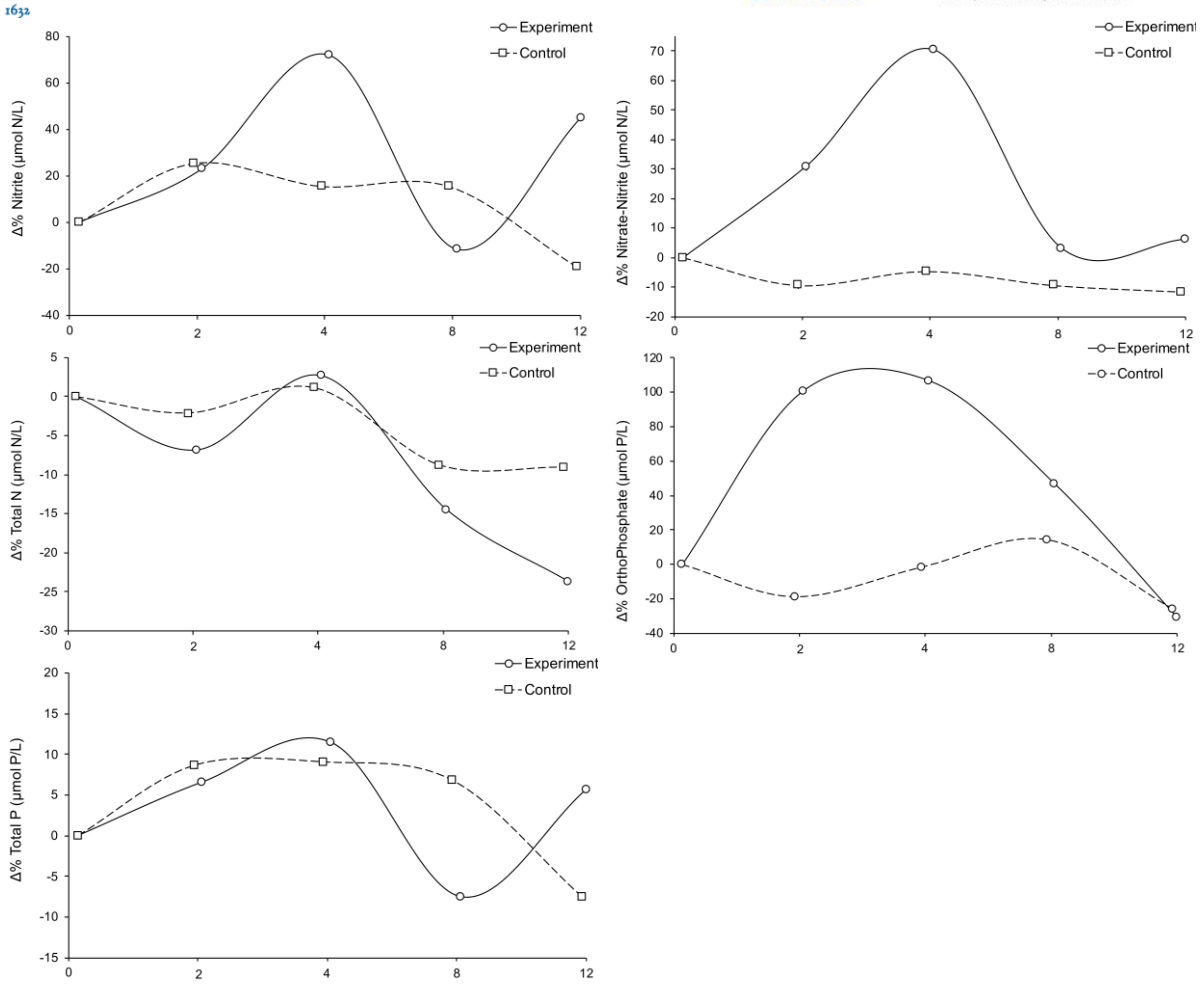

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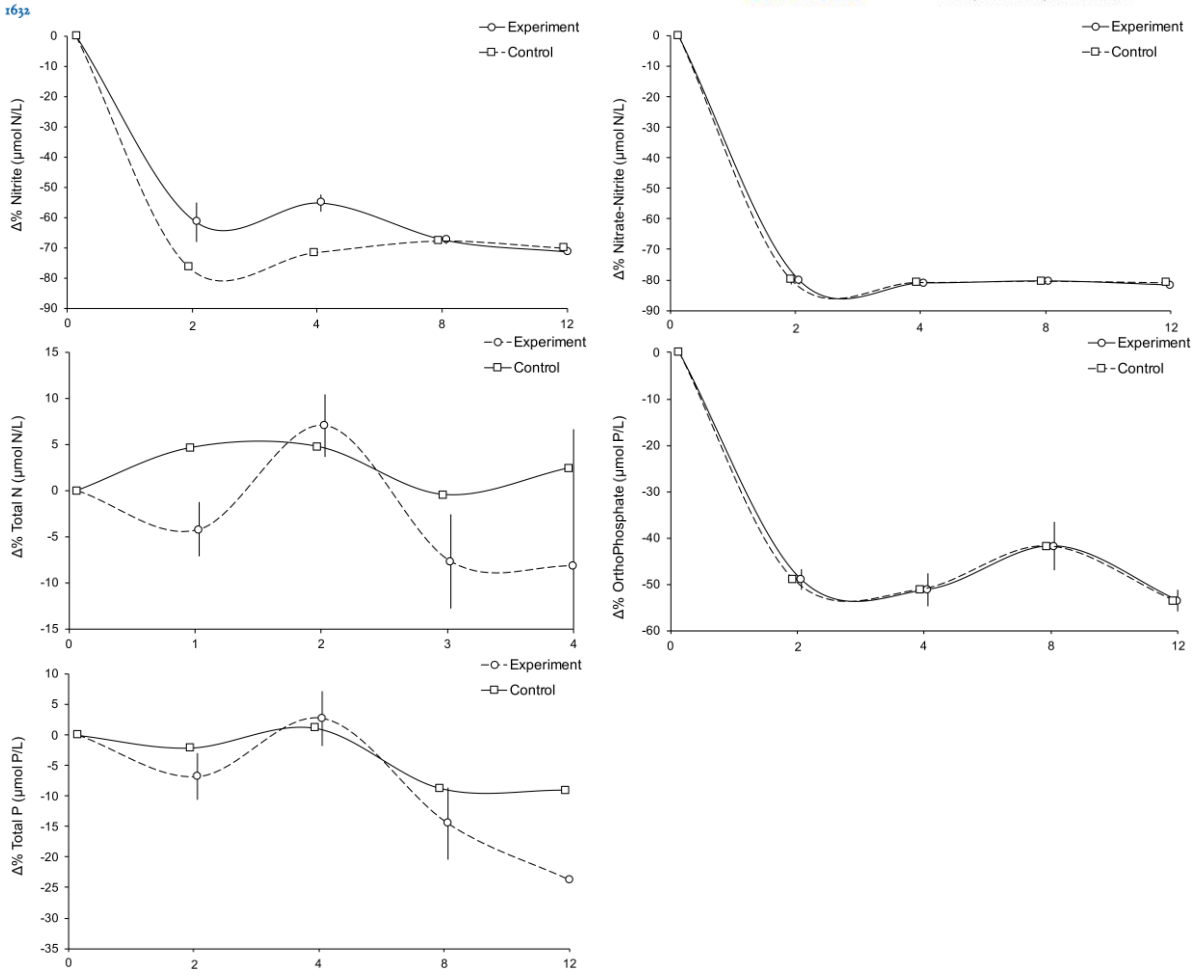

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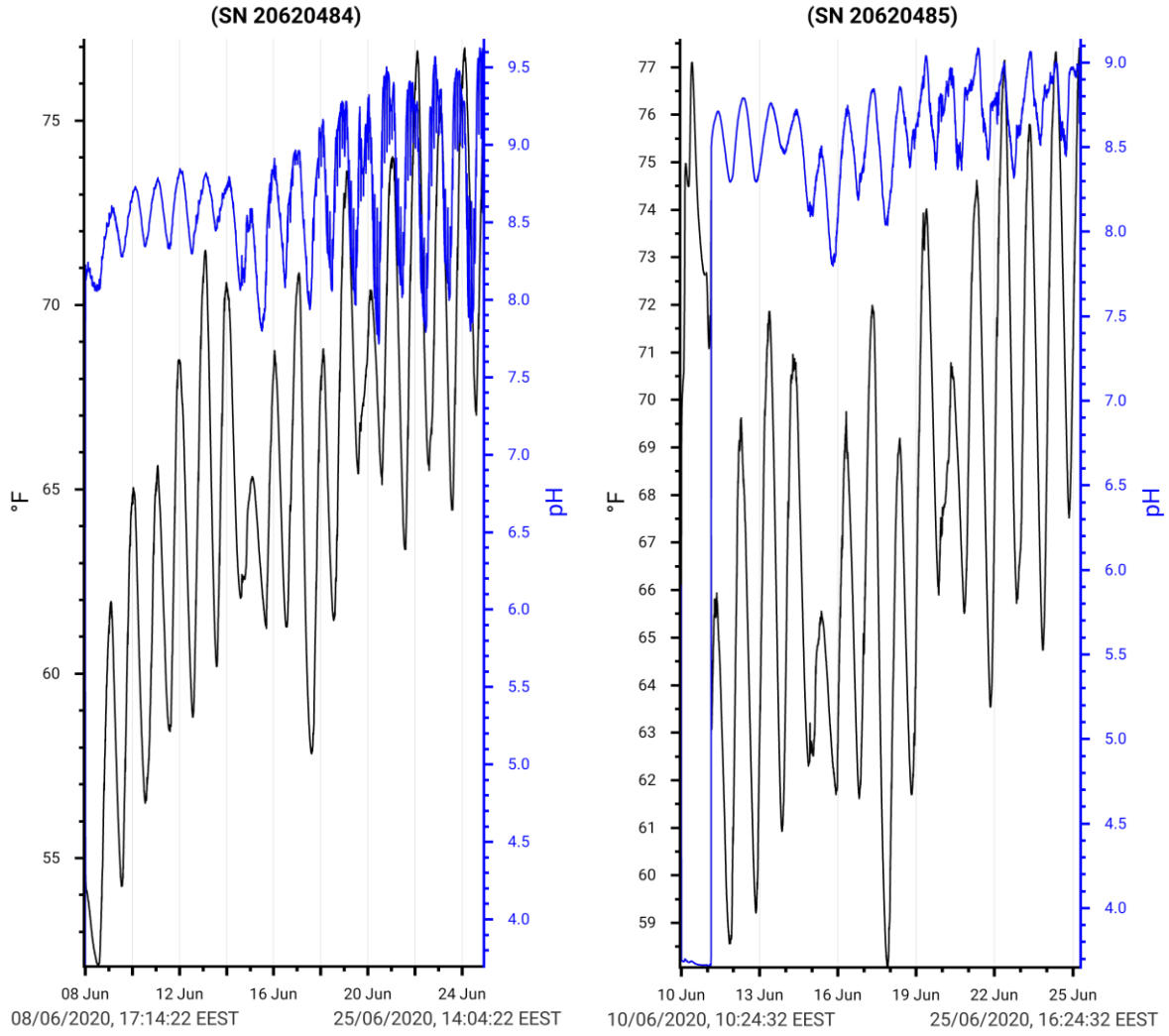
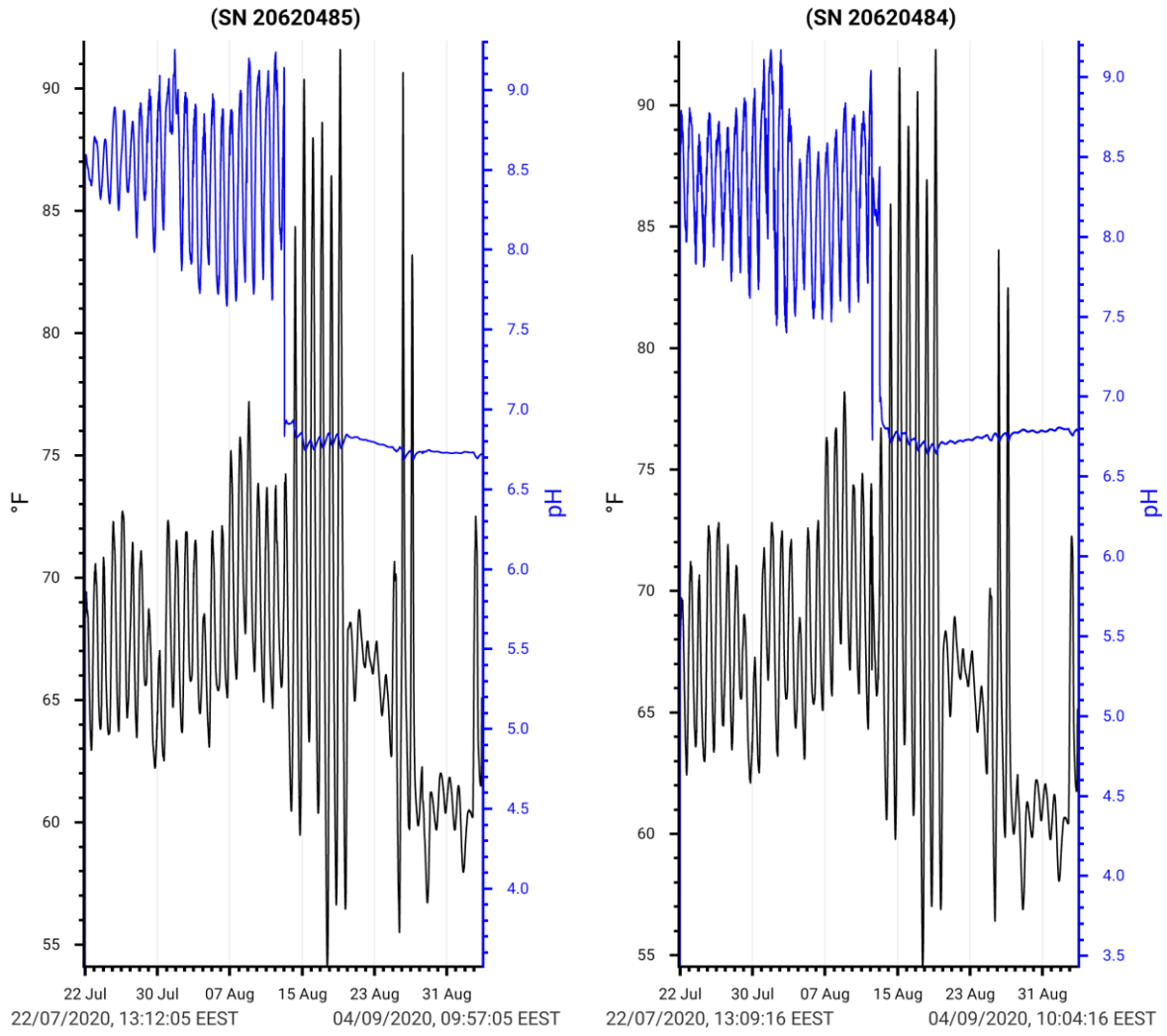
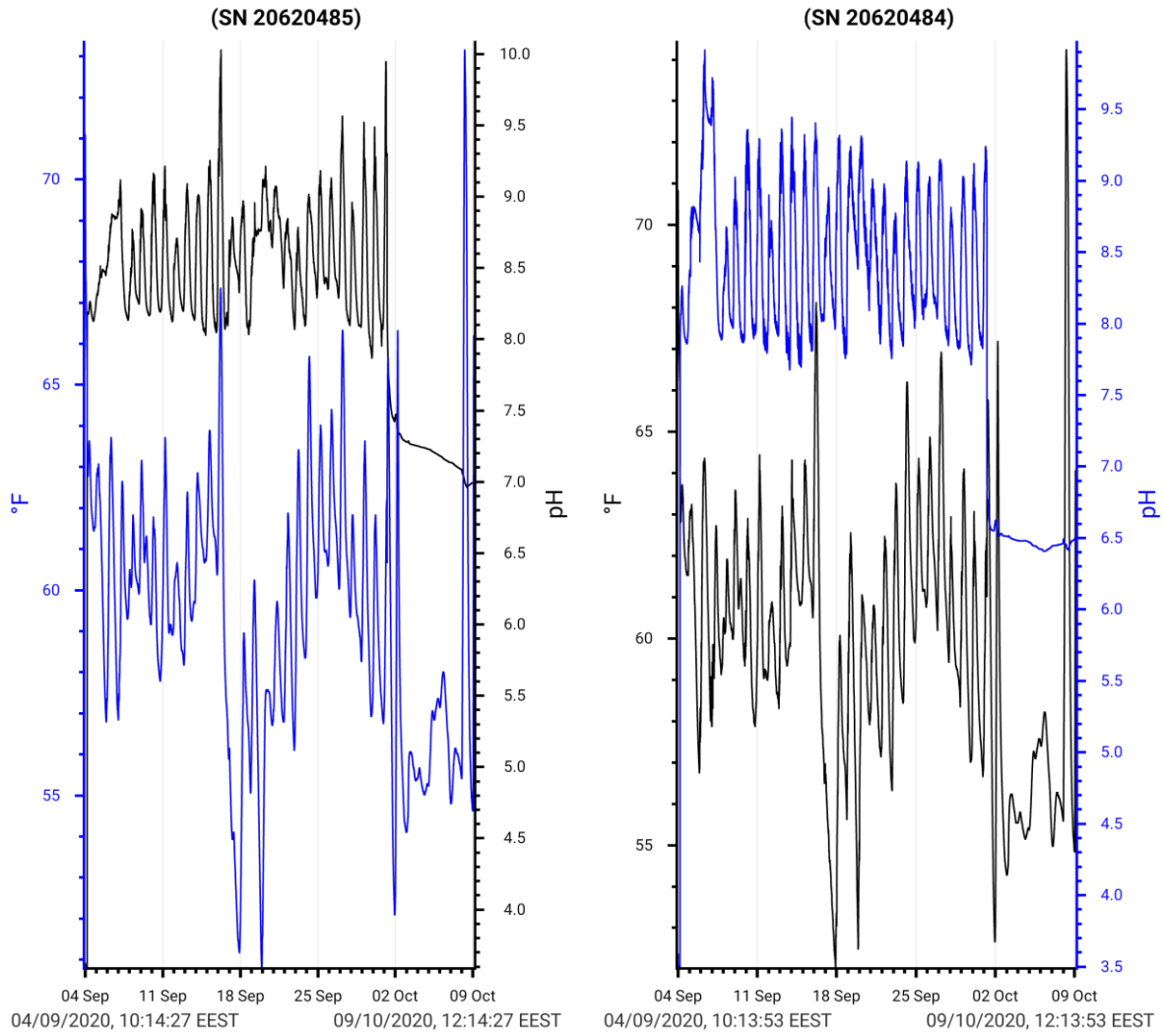
LISA 3



Joonis 1. pH ja temperatuuri anduri logi 2020. aasta esimese eksperimendi käigus (SN 20620485 – mahuti 4.6, kontroll; SN 20620484 – mahuti 1.6; *Ulva intestinalis*).

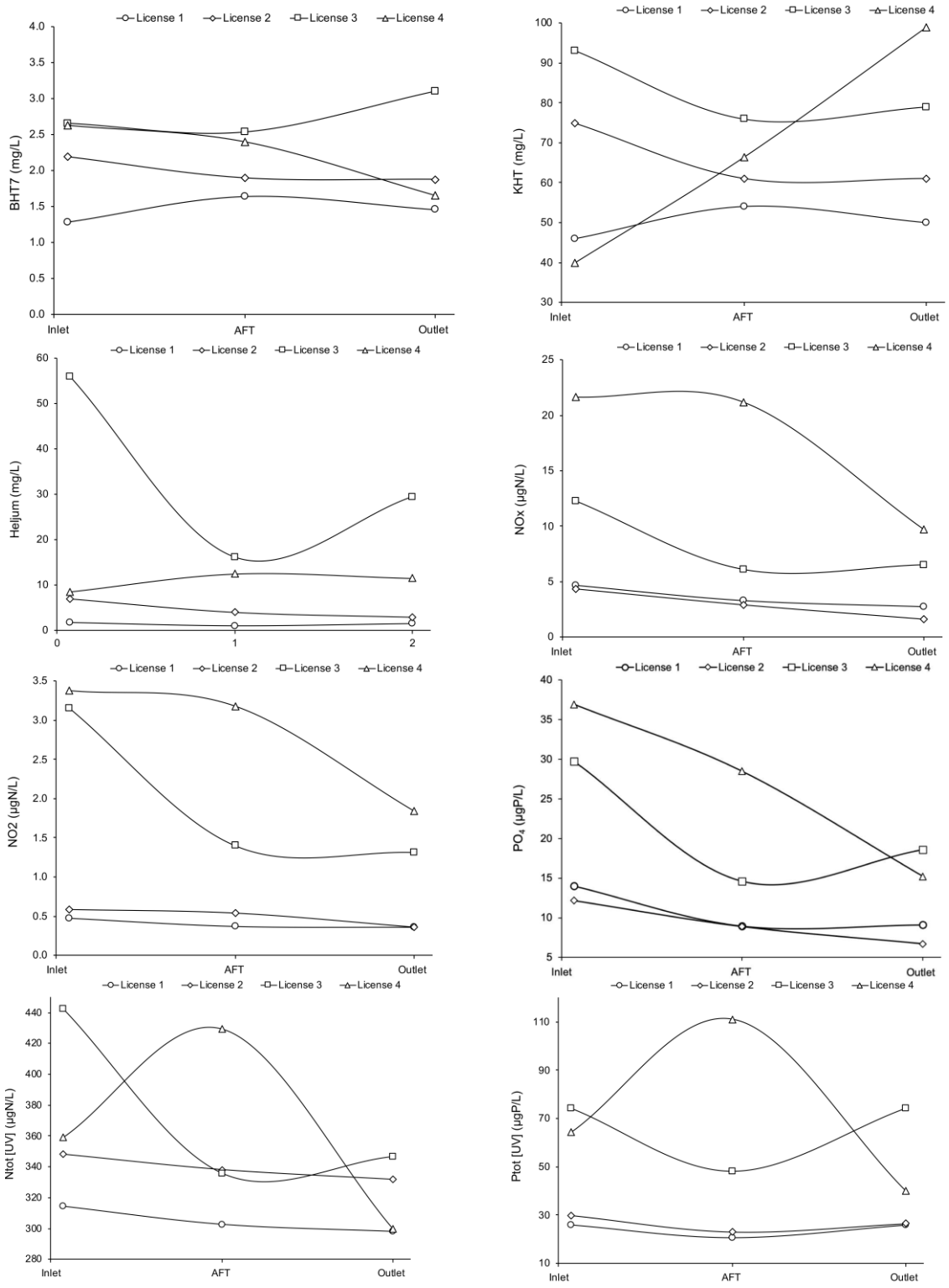


Joonis 2. pH ja temperatuuri anduri logi 2020. aasta teise eksperimendi käigus (SN 20620485 – mahuti 4.6, kontroll; SN 20620484 – mahuti 1.6; *Ulva intestinalis*).



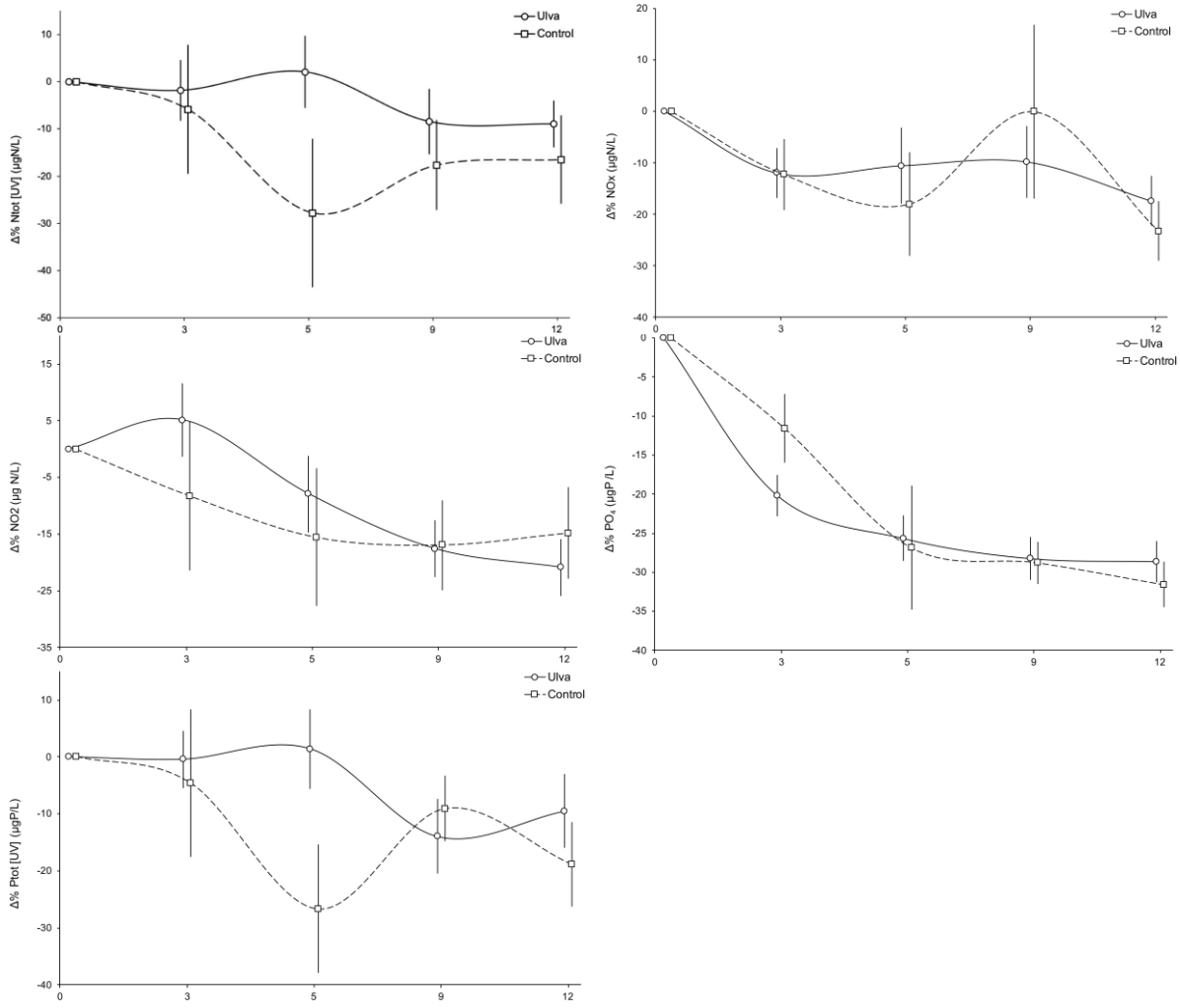
Joonis 3. pH ja temperatuuri anduri logi 2020. aasta kolmanda eksperimendi käigus (SN 20620485 – mahuti 4.6, kontroll; SN 20620484 – mahuti 1.6; *Ulva intestinalis*).

LISA 4

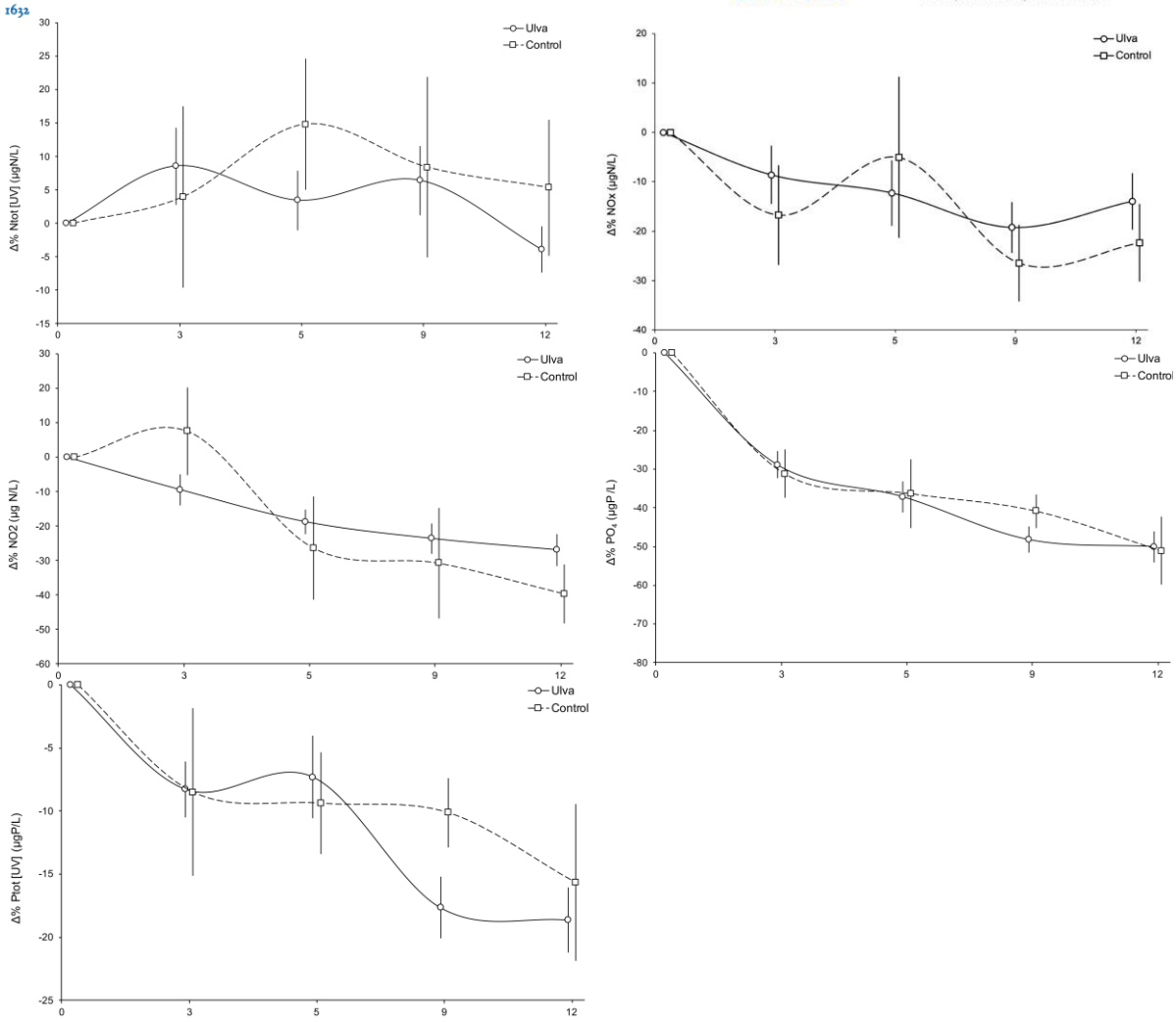


Joonis 1. 2020. aasta vee erikasutusloa seire tulemused. (License 1 - 8/06/2020; License 2 - 23/07/2020; License 3 - 31/08/2020; License 4 - 24/09/2020). Inlet – vee sissevõtt, AFT – pärast jämefiltrit, Outlet – vee väljavool süsteemist.

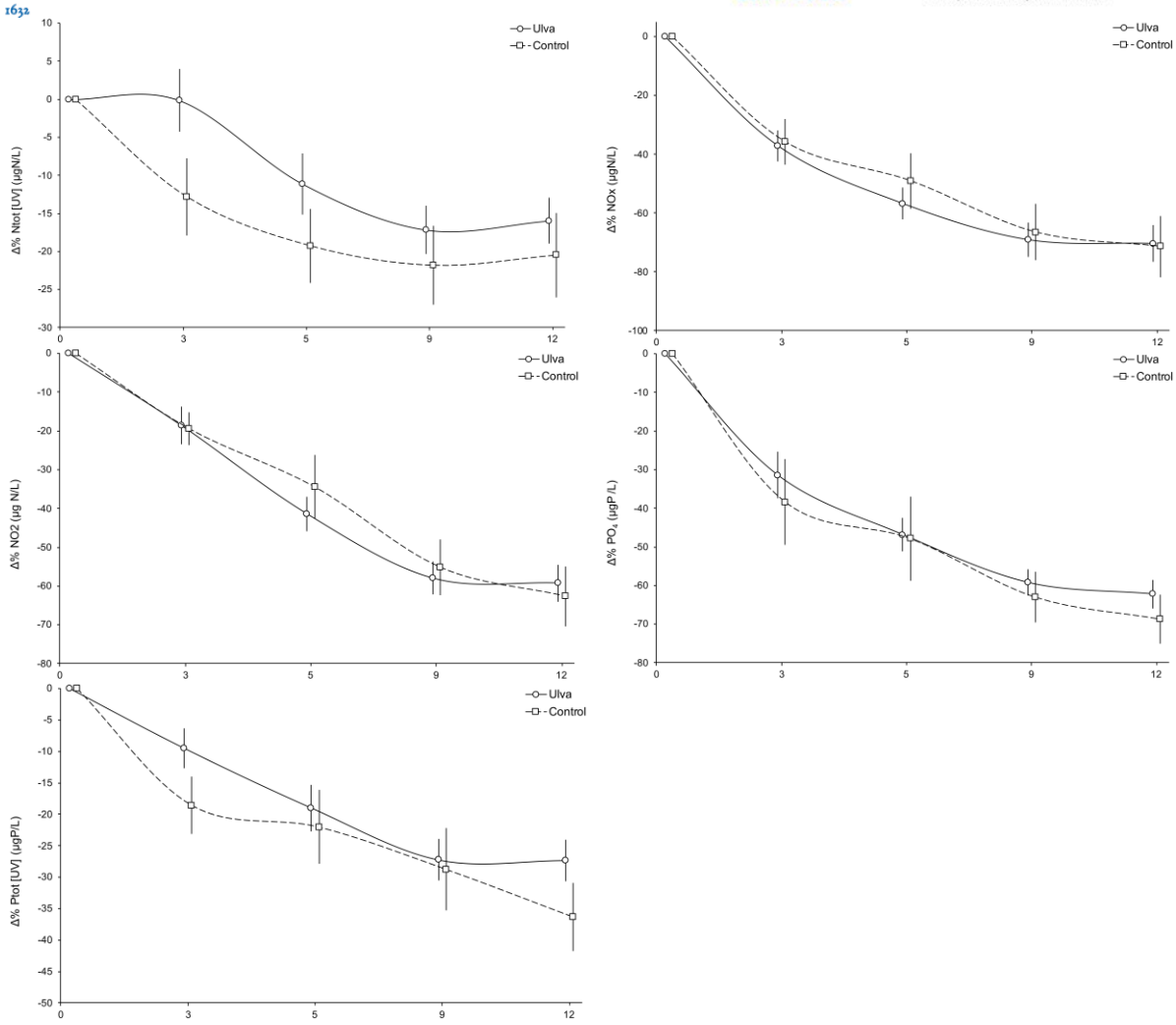
LISA 5



Joonis 1. Toitainete kontsentratsiooni muutus biofiltersüsteemis 2020. aasta esimese eksperimendi käigus (kogu andmetsik). X – teljel märgitud mahutite rea number.



Joonis 2. Toitainete kontsentratsiooni muutus biofiltersüsteemis 2020. aasta teise eksperimendi käigus (kogu andmetsik). X – teljel märgitud mahutite rea number.



Joonis 3. Toitainete kontsentratsiooni muutus biofiltersüsteemis 2020. aasta kolmanda eksperimendi käigus (kogu andmetsik). X – teljel märgitud mahutite rea number.

OÜ Eesti Keskkonnauuringute Keskus | Tartu osakond
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ANALÜÜSIAKT TA21000555 - TA21000560 - Taimne materjal (vetikamaterjal)

Tellja: **TÜ Eesti Mereinstituut**
Mäealuse 141, 12618 Tallinn

Proovivõtu aeg: 20.08.2020
Proovivõtja: Martin Georg; TÜ Eesti Mereinstituut
Proovivõtukoht: Saaare maakond, Saaremaa vald, Kehila küla, TÜ Eesti Mereinstituut, eksperimentaalne vetikakasvatus, Kesknõmme

Laborisse tulek: 02.03.2021
Analüüsi lõpp: 11.03.2021

Akti nr.	Proovi märgistus	Arseen (As) mg/kg KA STJnrMU94A	Kaadmium (Cd) mg/kg KA STJnrMU94A	Kroom (Cr) mg/kg KA STJnrMU94A	Mangaan (Mn) mg/kg KA STJnrMU94A	Plii (Pb) mg/kg KA STJnrMU94A	Tsink (Zn) mg/kg KA STJnrMU94A	Vask (Cu) mg/kg KA STJnrMU94A	Elavhõbe (Hg) mg/kg KA STJnrMU844A
TA21000555	13.08.20 T1.4	2,2	0,076	0,68	240	3,6	33	11	< 0,02
TA21000556	13.08.20 T1.5	2,3	0,069	0,50	450	4,4	30	15	< 0,02
TA21000557	13.08.20 T1.6	2,5	0,065	1,0	710	8,3	28	14	0,028
TA21000558	01.10.20 T1.4	4,8	0,094	5,5	740	400	48	46	0,021
TA21000559	01.10.20 T1.5	3,2	0,072	1,8	390	9,6	32	19	< 0,02
TA21000560	01.10.20 T1.6	4,6	0,061	2,3	750	12	39	35	0,024

Kommentaar: Proovid mineraliseeritud mikrolaineahjus.

Kinnitas: Tartu osakonna juhataja Hille Allemann/ 12.03.2021

LISA 7

Cultivation and Uses of Macroalgae

Literature review

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2021

Contents

Introduction

Macroalgae, more commonly known as seaweed, form a highly diverse group of photosynthetic organisms. Benthic in nature, they are found ubiquitously distributed along coasts, ranging from polar to tropical regions (Ferdouse *et al.*, 2018). Analogous to that of terrestrial plants, macroalgae constitute the basis of marine food chains acting to support countless other aquatic organisms through the formation of complex three dimensional habitat (Ferdouse *et al.*, 2018). Inhabiting both subtidal and intertidal zones, macroalgae contain specialty photosynthetic pigments utilized in photosynthesis.

Macroalgae are grouped into three distinct divisions: Ochrophyta-Phaeophyceae (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae), collectively, containing thousands of species each. Whilst highly productive, macroalgae are also known to produce a vast diversity of chemical compounds including primary metabolites such as carbohydrates, lipids and proteins to, and perhaps of greater interest, secondary metabolites, bioactive compounds unique to macroalgae that have potential applications in many commercial and research fields (Leandro *et al.*, 2019)

Macroalgae have a long history of exploitation by peoples all around the globe (Periera, 2016). Primarily utilised as a food source, edible seaweed provides a good source of proteins, lipids and dietary fibres when consumed by humans (Sánchez-Machado *et al.*, 2004; Dawczynski *et al.*, 2007; Macartain *et al.*, 2007). In more recent times, macroalgae have been recognised as an invaluable source of raw material to supply bioactive compounds for applications in food, animal feed, agriculture, cosmetics, pharmaceutical and biotechnological industries. These compounds are often found to be unique in nature and specific to certain macroalgal groups or species. Moreover, numerous studies have demonstrated the nutraceutical, pharmaceutical and cosmeceutical effect macroalgal derived bioactive compounds possess. Some these effects have the ability to mediate many medical symptoms, with properties related to anticancer, antiviral, antifungal, antidiabetic, antihypertensive, immuno-modulatory, cytotoxic antibiotic, anticoagulant, anti-inflammatory, anti-parasitic, antioxidant and UV-protection (Leandro et al., 2019; Stengel et al., 2011; Francisco et al., 2001; Smit, 2004; Dhargalkar & Verlecar, 2009; Mayer et al., 2013; Yuan & Athukorala, 2011; Pereira, 2008; Ruan, 2018). As such, macroalgae

have shown important potential in many medical treatments and researchers continue to investigate their pharmacological uses.

Aside from their chemical properties, macroalgae also provide many important ecological services. Large macroalgae are structuring species, acting to alter the environment in coastal zones by altering light regimes, sedimentation rates and effecting the overall hydrodynamics of a site (Reisewitz *et al.*, 2006; Leclerc *et al.*, 2013; Smale *et al*., 2013; Bertocci *et al.*, 2015). Furthermore, macroalgae are often a keystone species acting as a foundation for coastal food webs, providing complex three-dimensional habitat, food and settlement sites for juveniles of countless species (many of which are of important commercial or conservational importance). As a consequence, macroalgae support a large degree of coastal biodiversity.

Macroalgae's high photosynthetic productivity also implicates it as an important source of carbon storage globally. As macroalgal material is sequestered into sediments and exported into the deep ocean, it locks away atmospheric CO_2 and acts as a carbon sink (Gao & McKinley, 1994). Additionally, collecting or cultivating macroalgae for use in the production of fuels can act to offset anthropogenic atmospheric carbon production from fossil-based fuels by providing an alternative fuel source in the form of carbon neutral biofuels and bio-butanol (Enquist-Newman *et al.*, 2014; Kraan *et al.*, 2013; Potts *et al.*, 2012; Wei *et al.*, 2013. In addition to the capture of CO_2, macroalgae uptake dissolved inorganic nutrients such as nitrogen and phosphorous. This process stimulates algal growth and is important in mediating the deleterious effects eutrophication has in coastal zones, which as it stands, represents a major issue for many coastal regions around the globe (Leandro, 2019).

Macroalgae are photosynthetic organisms by nature. As such, they must generate compounds that absorb ultra-violet light as well as ones that protect from its damaging effects. These compounds come in the form or carotenoids, terpenes and phenolic compounds. As a consequence, they are useful photo-protective chemicals which are utilised in the production of commercial sunscreens (Guillerme *et al.*, 2017). Furthermore, macroalgae derived bioactive compounds are often extracted for uses in the cosmetics industry providing a useful source of colouring agents and stabilizers/emulsifiers for skincare products (Pimentel *et al.* 2017). With such a diversity of useful bioactive compounds and the potential for numerous novel

applications, macroalgae represent an important sustainable resource, leading to an increased demand for their study and exploitation.

As recognition for the importance macroalgae have in terms of the biological services they provide increases, and as demand for the products they produce grows globally, so does the interest the interest in their study and cultivation. As such, numerous stake holders with differing vested interests seek to invest in algae aquaculture production as a means to fill different sectors needs and wants. This comes at a time when the need for renewable resources that do not occupy declining arable land space or compete for resources such as freshwater or agriculture fertilisers, has never been greater (Ashkenazi & Israel; 2019). Thus, macroalgae aquaculture represents a significant opportunity to mitigate anthropogenic environmental effects, support diversity while simultaneously offering major economic benefits throughout numerous industries.

Macroalgae Cultivation

Historically, the Asia region has been the centre for the development of macroalgae cultivation and farming techniques but in more recent years significant developments have been made in both North America and Europe as interest in the practice has grown (Kim *et al.*, 2017; Ferdouse *et al.*, 2018). Traditionally, the large-scale cultivation of macroalgae in Asia has been for the the primary purpose of producing food as a resource. However, more recently in Europe and other parts the of the globe macroalgae farming has represented innovation with macroalgae servicing many novel applications (García-Poza *et al.*, 2020).

The global annual production of macroalgae has continued to increase year-on-year totalling more than 31 million tons (fresh weight) in 2016 (Ferdouse *et al.*, 2018). Of this, only 3.5% is produced from natural wild harvest methods compared to 96% produced by aquaculture operations representing roughly 27% of total global aquaculture output (Ferdouse *et al.*, 2018). Asia dominants production with China, Indonesia and other Asian countries representing 48%, 38.7% and 12.8% of total global production respectively (Goeke *et al.*, 2020). Macroalgae production within the Asian region is purposed primarily for human food consumption and as a food additive (Goeke *et al.*, 2020). Total global aquaculture has expanded at a rapid rate more

than doubling in the last 20 years (Ferdouse *et al.*, 2018) with estimates of 1000-100,000 million of tons to be produced.

Whilst macroalgae aquaculture is well established within Asia, developments are being made in the form or pilot studies and pre-commercial aquaculture operations for select brown and red algae of high economic value outside of the region. Such operations can be found in Europe (Callaway *et al.*, 2012; Hughes *et al.*, 2012; Peteiro *et al.*, 2016; Stévant *et al.*, 2017); South America (Buschmann *et al.*, 2014; Camus *et al.*, 2016; Pellizari & Reis 2011) the USA (Augyte *et al.*, 2017) as well as parts of Africa (Msuya, 2011). In contrast to Asian operations where there is a high demand for macroalgae as a food product, new operations typically target the production of biochemical products destined for Western pharmaceutical industries seeking novel high value products (Hafting *et al.*, 2015).

Major Cultivated species

As global demand for seaweed products grows the volume and diversity of seaweed species cultivated has increased markedly. As it stands currently more than 200 macroalgae species are cultivated globally for commercial use. However, of this number only a select few are intensely cultivated. The main genera cultivated include; *Saccharina* and *Undaria* (brown algae); *Porphyra, Pyropia, Eucheuma/Kappaphycus* and *Gracilaria* (red algae); and *Monostroma* and *Enteromorpha/Ulva* (green algae) (Kim *et al.*, 2017; Lüning & Pang, 2003). Figure 1 shows some of the most cultured seaweed worldwide such as *Eucheuma* spp., *Saccharina japonica* (formerly *Laminaria japonica*) and *Gracilaria* spp.

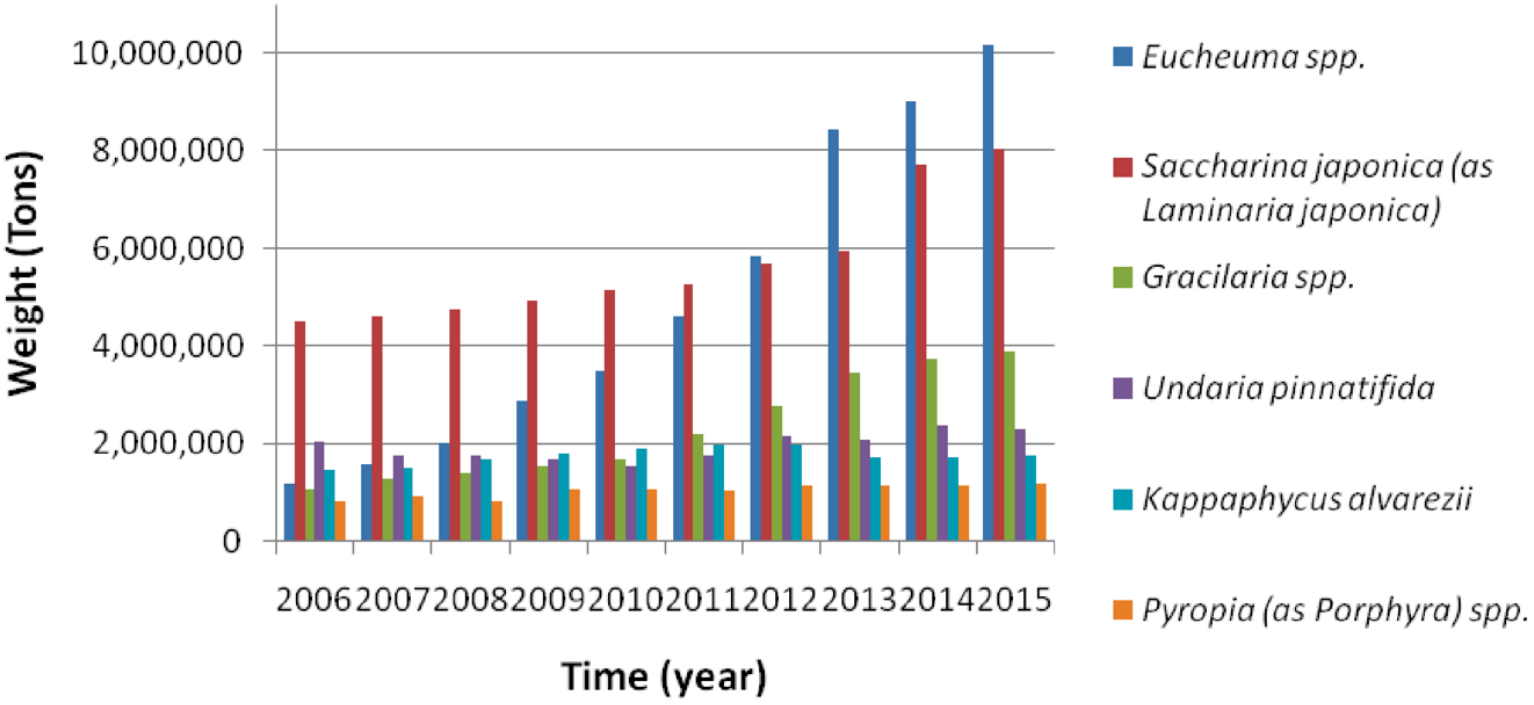

Figure 1. Main seaweed species cultured globally, in tons. Adapted from FAO—The global status of seaweed production, trade and utilization, (Ferdouse *et al.*, 2018).

Requirements for Macroalgae Cultivation

Macroalgae cultivation is primarily dependent upon seawater containing sufficient nutrients to act as a growth medium. As a photosynthetic organism, macroalgae growth rates are determined upon environmental factors such as temperature, nutrient availability, pH, CO_2, solar radiation and salinity (Dawes *et al.*, 1998; Choi *et al.*, 2010; Guo *et al.*, 2015). However, such factors combine in a complex interplay to determine a given growth rate dependent on the macroalgae species under cultivation, of which each species is unique. Furthermore, as many algal species display complex and poorly understood life stage histories, the factors that control both germination and growth likely change through time adding to the complexities of cultivation and maximising production (Cumming *et al.*, 2019).

Phaeophyceae (Brown Seaweed)

Phaeophyceae or Brown algae are a large group of photosynthetic multicellular organisms comprising of over 1500 species and with a temperate, circum-polar distribution. Alongside chlorophyll, phaeophyceae are characterised by the chemical compound fucoxanthin which is produced in different compositions and concentrations among different brown algae species. Fucoxanthin has been shown to possess numerous therapeutic qualities including antitumoral antioxidant and anti-obesity properties (Yan *et al.*, 1999; Maeda *et al.*, 2005; Mise *et al.*, 2011; Pigmen *et al.*, 2014).

Alginate is a naturally occurring polysaccharide found within the cell walls of Phaeophyceae (García-Poza *et al.*, 2020). Comprised of varying chemical structures, alginate displays different characteristics based upon the genera it is derived from. *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis* and *Sargassum* spp. are brown species all known to produce alginate compounds. Other sources of alginate include *Eklonia radiata, Saccharina japonica and Undaria pinnatifid,* all of which are cultivated primarily for human consumption. Furthermore, *Laminaria hyperborea, Laminaria digitata, Saccharina japonica, Ascophyllum nodosum, Ecklonia maxima, Macrocystis pyrifera, Durvillea antarctica, Lessonia nigrescens* and *Lessonia trabeculata* are large brown species that are typically utilised in the production of alginate although they are usually restricted to shore gathering rather than cultivation. Alginate as a product has been extensively investigated for both commercial and therapeutic uses. Utilised in food products, cosmetics, textiles as well as numerous biomedical applications, alginate has the properties of being an emulsifier, binding agent and the ability to condense aqueous solutions to form gels and as such is found throughout a range of industries (Wiltshire *et al.*, 2015; Imeson, 2009)

Laminarin is another naturally occurring polysaccharide derived from brown macroalgae that is of important commercial use. The molecule Laminarin acts as a storage glucan and is utilised as the main energy reserve by *Laminaria* spp. Dependent upon the species, harvesting method, season and habitat, Laminarin can represent over 35% of brown algae total biomass by dry

weight. Furthermore, the composition of Laminarin is also modified by abiotic factors such as wave motion, salinity, depth and temperature as they function to influence physiological activity (Rioux *et al.*, 2010). As a percentage of dry weight, Laminarian content has been reported to be 0–33% for *Saccharina latissimi*, 0–32% for *Laminaria hyperborean* (Holdt & Kraan, 2011), 14% for *Laminaria digitate*, 3% for *Undaria pinnatifida*, 4.5% for *Ascophyllum nodosum* (Macartain *et al.*, 2007) and 84% for *Fucus vesiculosus* as a percentage of total sugar content (Rioux *et al.*, 2010). Laminarin extracted from Phaeophyceae has the potential to be used within a diverse range of products with applications in immuno-stimulation, antitumor, epidermal wound healing (Kadam *et al.*, 2015) and may act as a stimulator for regulating intestinal metabolism (Devillé *et al.*, 2004; Devillé *et al.*, 2007). Furthermore, laminarin's biochemical properties have been demonstrated to mediate irradiation sickness, reduce cholesterol (Holdt & Kraan, 2011). Whilst further research is likely needed for the further development of such applications, laminarian stands to be a highly valuable commercial product.

Fucoidan, a complex polysaccharide found within the cell walls of certain phaeophyceae is another compound found to possess useful bioactive properties. Fucoidan is distinguished by its structure which is comprised of fucose containing sulphated polysaccharides and represents a diverse set of structurally related compounds. Fucoidan is typically found in concentrations that warrant extraction from brown algal species such as *Fucus vesiculosus*, *Sargassum aquifolium*, *Saccharina japonica* and *Undaria pinnatifida*. As the structure of fucoidan is complex and its formation linked to numerous physiological factors, fucoidans are found to be species distinct (Li *et al.*, 2008). Fucoidan is noted for its numerous bioactive properties and is utilised throughout the medical and pharmaceutical fields. Fucoidan acts an anticoagulating agent as well as processing antiviral and antioxidant capabilities (Mandal *et al.*, 2007; Chandía & Matsuhiro, 2008). Moreover, fucoidan is utilesde extensively within the skincare industry with alleged moisturizing, anti-aging and anticellulite properties and represents a significant source of commercial activity (Chizhov *et al.*, 1999; Wijesinghe & Jeon, 2012; Peng *et al.*, 2013).

Chlorophyta (Green)

Chlorophyta or green algae so called due to the chlorophyll (a and b) pigments that give its appearance form a large group of photosynthetic organisms. Chlorophyta utilise these pigments along with carotenoids, not only for energy production but also to protect the damaging effects of ultra-violet light (Barsanti & Gualtieri, 2006) and as chemical defence (Kadam *et al.*, 2013).

Chlorophyta have been shown to be a rich source of carbohydrates, particularly that of sulphated polysaccharide which are structured within the algal cell walls (Lahaye & Robic, 2007). One such polysaccharide, ulvan, derived from Ulvaceae is a water-soluble gelling polysaccharide with bioactive properties such as immunomodulating, antiviral, antioxidant and anti-cancer (Kidgell *et al.*, 2019). Ulvans account for roughly 20-30% of the total carbohydrate component of chlorophyta but their bioactive concertation and function vary dependent upon factors that pertain its given chemical structure. Therefore, ulvan bioactivity is highly diverse and differs based on the species from which it is extracted from as well as the environmental factors effecting an individual plant (Kidgell *et al.*, 2019). Ulvan is of interest to the biomedical industry, its potential use in applications related to tissue engineering, antibacterial biofilm prevention and as a drug delivery device have been noted by researchers once it was proven ulvan is recognised animal liver cells [Kidgell *et al.*, 2019; Alves *et al.*, 2013; Wijesekara *et al.*, 2011; Venkatesan *et al.*, 2015; Cunha & Grenha, 2016). The development of products related to such effects has the potential lead to significant economic opportunities.

In addition to ulvans unique gelling and bioactive properties, chlorophyta are reported to have novel uses outside of the food and pharmaceutical industries. Anionic polysaccharides found within *Ulva* sp. have the ability to accumulate heavy metals within the algal cell structure. As such, *Ulva* sp. can concentrate heavy metals found to pollute contaminated waters and when removed and destroyed can mediate pollution (Webster & Gadd, 1996; Bocanegra *et al.*, 2009; Schijf & Ebling, 2010). This ability by *Ulva* sp., therefore can be utilised in the mitigation of anthropogenic wastewaters as the species display high growth rates particularly under high nutrient regimes (Kraan, 2013; Castine *et al.*, 2013, Lawton *et al.*, 2013; Glasson *et al.*, 2017). Ulva propagation is therefore positioned as a useful tool for environmental managers for heavy metal bioremediation.

Overall chemical compounds derived from chlorophyta have been demonstrated to be highly diverse in nature with applications in pharmaceuticals, nutraceuticals, foods, feed, agriculture and bioremediation.

Rhodophyta (Red algae)

Representing one of the oldest groups of eukaryotic algae, Rhodophyta (Red algae) are named after the so called phycocyanin and phycoerythrin pigments which give the algae its typical red appearance (Kadam, 2013; Knowler *et al.*, 2020). These pigments are proven to be highly useful as natural commercial dyes utilised in products ranging from chewing gum, beverages, dairy products as well as cosmetic products including lipsticks and eyeliner (Spolaore *et al.*, 2006). Additionally, phycocyanin and phycoerythrin have medicinal properties with investigations demonstrating anti-oxidative, anti-inflammatory, anti-viral, anti-tumor, neuroprotective and hepatoprotective activities (Sekar & Chandramohan, 2008).

One of the most widely used products derived from red algae is the polysaccharide Agar. Agar is present within the cell walls or certain red algae species, principally that of the orders Gelidiales and Gracilariales. *Gracilaria* spp. represents the main source of commercial agar extraction accounting for 80% of global production (Ferdouse *et al.*, 2018). The relatively easy extraction of agar from macroalgae represents an economically important raw material and has applications in numerous industries (Yarnpakdee *et al.*, 2015).

Methods of Cultivation

The cultivation of macroalgae is predetermined by the specific growth requirements of a given algal species. In general, the physical properties of seawater used as a cultivation medium are the main environmental factor regulating growth. Macroalgae growth is always regulated to varying degrees by the factors of temperature, pH, salinity, nutrient availability and solar radiation (PAR). Moreover, macroalgae often display complex lifecycles and as such certain environmental factors will affect algal growth disproportionality at varying life phases. Thus,

a high degree of biological and technical knowledge is required for a cultivation venture to succeed.

Macroalgae aquaculture can be conducted both onshore and offshore as well as integrated with other aquaculture farms such as fish known as integrated multitrophic aquaculture (IMTA).

Table 1. Main techniques of macroalgae cultivation.

	Onshore Methods	**Offshore Methods**
Line cultivation: **-Off-Bottom** **-Submerged hanging line** **-Floating Line (long-line)**	Yes	Yes
Net cultivation	Yes	Yes
Float raft cultivation	Yes	Yes
Tank or Pond cultivation	Yes	No
Rock based farming – direct planting onto substrate	No	Yes

Onshore Cultivation

Commercial onshore aquaculture practices began in the 1970s with the goal to produce carrageenan extracted from *Chondruscrispus* (García-Poza *et al.*, 2020). The most obvious advantage of onshore aquaculture is the ability to actively monitor algae growth factors thus allowing for the opportunity to make real-time adjustments to these factors (Hafting *et al.*, 2015). The infrastructure setup for onshore aquaculture typically takes place in closed systems comprising of either tanks, ponds, raceways or lagoons independently or in tandem with one another. Such systems usually require additional energy input in the form of agitation in order to keep seaweeds suspended and exposed to light (Hafting *et al.*, 2012; Currie, 2018). Water inflow and outflow can be closely monitored as water is brought into the site from external sources of water or from onsite aquafers being supplied as needed. This allows for the accurate

addition of nutrients enabling a tight and efficient control over the growth media (Hafting *et al.*, 2012; Reid *et al.*, 2020). Furthermore, the outflow of wastewater into the environment can be monitored for levels of pollutants minimizing impacts and enabling for obligations to environmental restrictions to be met. Additionally, the quantity and quality of light can readily be manipulated through a variety of methods (Spectrum, shading, intensity, tank depth, light duration/cycling) in order to optimise for growth and efficiency. Other water chemical factors such as CO_2 and pH can also be readily monitored and altered. Such an ability is useful as these factors are linked to algal biochemical compound production and therefore concentrations can be influenced. Salinity can be manipulated through means of altering freshwater and saltwater ratios or through the addition of salt crystals. Controlling abiotic growth factors helps and standardising culture conditions leads to higher quality final algal products obtained. Furthermore, algal stocking densities can be held at levels that maximise production increasing overall harvest yield in both slow and fast-growing species [Hafting *et al.*, 2015].

Overall, land based algal aquaculture systems demonstrate distinct advantages over offshore systems. Land based aquaculture allows for the cultivation of virtually all macroalgae genera and forms (excluding the largest of macroalgae forms) and as such products can be developed from species that may occur naturally in low densities. Onshore systems are also not affected by adverse conditions such as tides, waves and wind removing the pressures of certain grow seasons and windows of opportunity for work to occur. Onshore facilities also enable more readily the cultivation of macroalgae at a small scale as a means to test and refine growing methods and products before upscaling (Hafting *et al.*, 2012)

The high cost of onshore facilities represents the largest disadvantage for land-based aquaculture systems. High energy inputs required to run pumps, lighting and filtration systems combined with building construction and maintenance costs mean only highly profitable commercial operations are economically feasible outside of subsidised research initiatives.

Offshore Cultivation

The cultivation of macroalgae as both a food source and a source of chemical compounds onshore is limited to species of high economic value due to high operation costs. An alternative to this is the cultivation of seaweed using offshore infrastructure. While lacking any one distinct

definition, offshore aquaculture can be considered any farm of marine products located some distance from a coast and one that is principally situated in exposed environmental conditions.

Offshore farmed macroalgae can either be attached to a hard substrate situated on the seafloor, or more commonly on longlines and nets that are anchored to the seafloor whereby seaweed is seeded or attached as individuals (Currie, 2018, De Góes & Reis, 2011). Cultivation can also be carried out in multiple steps whereby young individuals are first cultured as seedlings indoors or in greenhouse tanks on land before being translocated onto ropes offshore (Peteiro *et al.*, 2014). Due to reduced infrastructure installation costs and less costly, labour-intensive maintenance relative to that of land-based systems, offshore farming offers an economically sustainable means of seaweed biomass production.

As promising as offshore cultivation is, it is not without its drawbacks and currently many challenges exist. In these farming systems major issues are presented by the nature of the growth environment wherein seaweeds are susceptible to adverse environmental conditions, particularly in the form of high energy waves. As such, farms must be designed in such a way to overcome these issues and the need arises to invest and develop solutions to meet these challenges while remaining financially viable. Hence, successful farm operations must carefully consider farm design and the materials that are used to withstand high energy seas (Fernand *et al.* 2017) . Typically, careful consideration is made in terms of the location of offshore aquaculture systems positioned in coastal zones such that waterways high in water movement are prioritised as a means to supply operations with inorganic nutrients (Harrison & Hurd, 2001).

A major problem that offshore macroalgae aquaculture operations can suffer from is that of fouling by macroscopic epiphytes such as bryozoans, other seaweeds, hydroids, gastropods and bivalves. These epiphytes work to induce deleterious effects on cultivated macroalgae often degrading algal tissue causing high biomass loss or slowing growth through increased competition for nutrients (in the case of epiphytic macroalgae). As such, in order to mitigate the challenges of biofouling, harvest must take place in late spring/early summer periods, limiting the growing season, reducing biomass and more importantly reducing the bioaccumulation of polysaccharides (Fernand *et al.* 2017). The issues presented by epiphytes and the limited nutrients available in open sea aquaculture means species selected for

cultivation must be robust enough to resist epiphytes while simultaneously adapted to local conditions. Furthermore, whilst space is typically less of a limiting factor for the expansion of offshore operations, changes in climate may act to alter water temperature and chemistry resulting in the potential for a reduction in regions suitable for macroalgae cultivation. (Troell *et al.*, 2017; Oyinlola *et al.*, 2018).

Despite the challenges associated with offshore aquaculture, recent years have shown increased interest in developing innovations to move offshore aquaculture operations further out to sea. However, in order to overcome the harsh environmental conditions associated with the open seas' developments in innovative and novel technologies in regard to both cultivation and harvesting is required.

Integrated Multitrophic Aquaculture

Traditional single species aquaculture whereby one species is cultivated in a manner that maximises biomass production is increasingly viewed as overly simplistic and one that contributes to environmental degradation of the marine environment. In order to mediate some of the environmental impacts associated with animal aquaculture, such as eutrophication from excess nutrients, the spread of disease, as well as improving farm output from a given area, seaweeds are being integrated into traditional animal aquaculture operations. The practice of co-farming multiple aquaculture species in close proximity is known as integrated multitrophic aquaculture (IMTA) and provides numerous benefits through the interconnection of species. The IMTA model prioritises cultivating species whose products (inorganic and organic) of one species are up taken by another to serve as an energy source. As such, the need for the addition of costly fertilisers to promote seaweed growth is reduced and profit is increased sustainably through seaweed biomass growth.

Several studies have assessed the effect fish aquaculture effluent and waste products has on the growth of macroalgae. These investigations found that seaweed biomass increased when cultivated within existing fish farms. A study by Buschmann *et al.*, (2008) demonstrated that seaweed grown in close proximity to salmon aquaculture operations in combination with other filter feeders in an IMTA arrangement resulted in the uptake of, and absorbance of, organic

and inorganic nutrients. Such an arrangement reduces the environmental impact of salmon farming operations (Buschmann *et al.*, 2008).

Integrating macroalgae production into current animal cultivation methods may also benefit farm operations through bioremediation and other biological services. As macroalgae grow they uptake excess nutrients from the water column providing a filtering effect improving overall water quality and offsetting detrimental farm effects. Furthermore, macroalgae cultivation can offset environmental impacts on land. Through their use as a fertiliser to improve soil condition and substituting synthetic chemicals macroalgae can offset atmospheric emissions. The environmental benefits of macroalgae aquaculture are therefore felt both at a local and global scale with the mitigation of eutrophication and increased support of biodiversity acting locally, and carbon sequestration or 'blue carbon' acting globally (Hossain *et al.*, 2008; Duarte *et al.*, 2013). With this in consideration aquaculture operations can make use of environmental tax subsidies to improve their economic viability.

One of the greatest challenges with implementing IMTA into traditional single species aquaculture operations is identifying suitable seaweed species for culture. Typically, species high in productivity/growth rates i.e. high nutrient uptake, high in economic value and that are relatively hardy in regard to environmental conditions are most suitable for IMTA. By optimising farm design and utilising data driven models combined with primary biological research seaweed species can be selected for IMTA to optimise economic gain and environmental mediation.

By adopting IMTA practices, aquaculture operations have the ability to not only reduce their environmental impacts, but also gain economic benefit by diversifying products that can be commercialised and brought to market. Figure 2 provides an example of an IMTA operation.

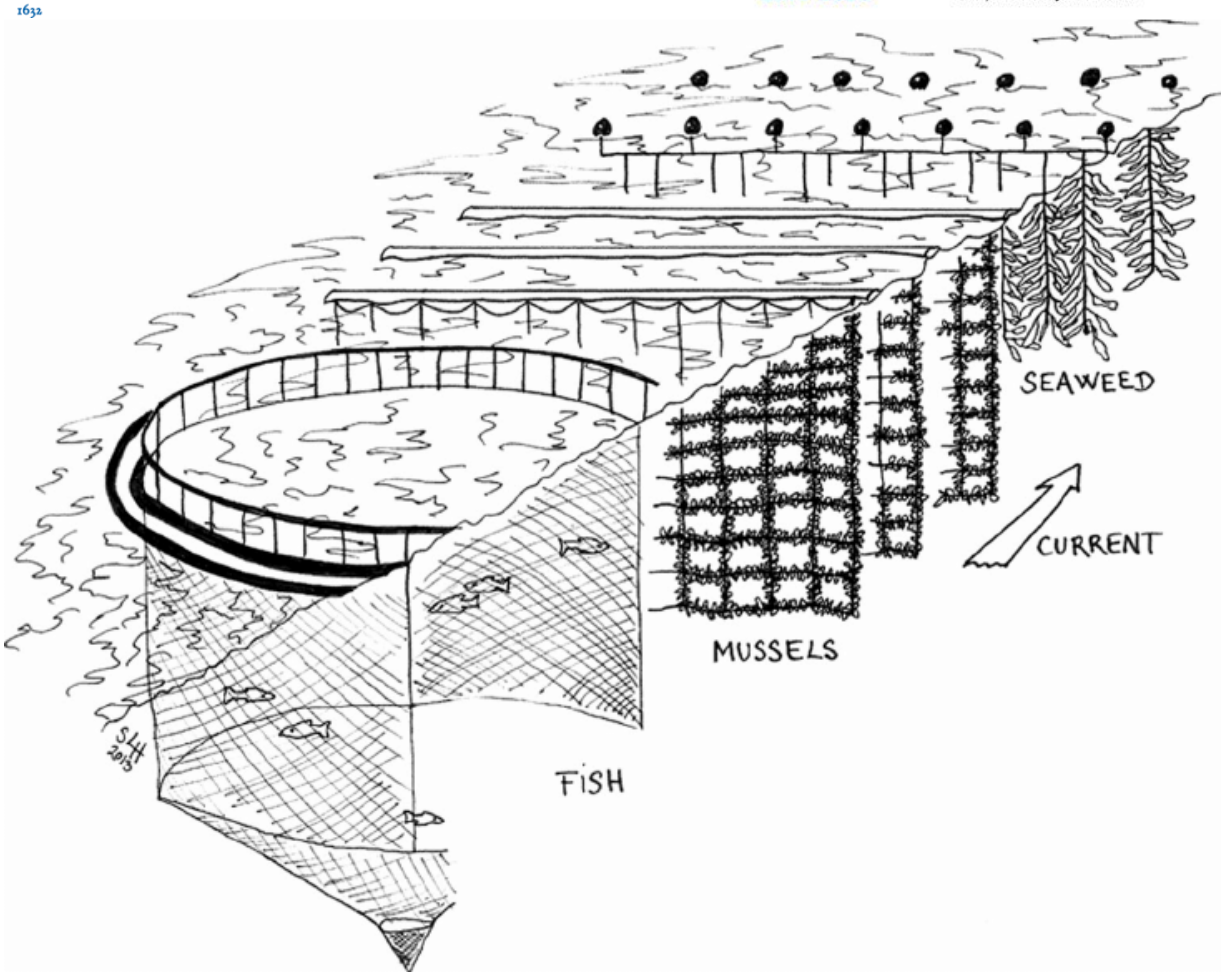

Figure 2. Schematic of an integrated multi-trophic aquaculture (IMTA) example of rainbow trout in a polar circle cage, mussels on a SmartFarm TM longline and seaweed suspended on droppers on longlines (Holdt & Edwards, 2014).

Future Research and Challenges

Macroalgae cultivation is of significant economic importance to many regions of Asia and its development within the European, North America and Oceania regions signals large opportunities for growth. As a renewable resource, seaweed aquaculture represents a sustainable method of production of natural compounds for product areas such as cosmetic, therapeutics, pharmaceuticals biopolymers, food. Seaweed aquaculture also has the potential to act as an important carbon sink, sequestering atmospheric CO_2 as a means to offset anthropogenic climate change (Sulaiman *et al.*, 2012). As the global demand for macroalgae

and its associated products grows the need for the expansion and improved optimisation of seaweed aquaculture is needed to meet these demands.

One of the main drivers for advancements within the field of macroalgae aquaculture is the collaborative work between academia and the aquaculture industry. Collaboration between these groups has resulted in research and development programmes culminating in several research initiatives which aim to improve growth efficiencies, develop aquaculture systems, advance primary biological knowledge as well as better integrate ideas in relation to sustainability and the 'blue economy' (Hafting *et al.*, 2012).

At present there is a strong need for the optimisation of cultivation techniques in regard to the onshore production of macroalgae (Hafting *et al.*, 2012; Hafting *et al.*, 2015, Sulaiman *et al.*, 2012]. Moreover, existing offshore aquaculture infrastructure is restricted to sheltered waters. As such, cultivation in deep water or open seas is not possible due to increased mechanical forces. As a result, there remains large knowledge gaps for both onshore and offshore aquaculture in terms of developing cultivation systems that prove to be economically viable and environmentally sustainable. In order to overcome these challenges a concerted effort needs to be placed into researching both the abiotic and biotic factors that control for production in order to stabilise production processes.

In terms of macroalgae production, growth rates are directly linked to their surrounding growth medium. Such a fact represents a distinct advantage in regard to research and development as it allows researchers to modify abiotic factors to directly influence growth rates. This serves as one of the main advantages of onshore research. One key aspect that is lacking is knowledge of how abiotic factors such as light, pH, salinity, water motion, conductivity and nutrient concentration interplay with one another to produce a given macroalgal growth rate. Such data is important to understand as it underpins the economic feasibility of a given commercial aquaculture operation.

As the cultivation of macroalgae increases globally so does the volume of biomass produced. However, not all biomass is equal and depending upon the method in which this biomass is obtained, its use may not be suitable for all applications. Moreover, many countries operate strict health laws governing how food may be cultivated, this combined with licensing and regulations may act to restrict the ways in which macroalgae biomass can be utilised. For example, the application of macroalgae to land as a fertiliser in agriculture typically encounters fewer regulations and oversights relative to macroalgae biomass produced for human consumption which is subject to more strict governance. Furthermore, other uses for biomass such as a feedstock for fuel production or as an animal feed addictive, which depending on the country and industry, may or may not encounter strict regulations. As a consequence, macroalgae biomass, must be evaluated in terms of the species and method in which it is produced, the regulations that apply to it, as well as the technically difficulties associated with utilising that biomass for a given application in order to ascertain is most economically viable use.

Macroalgae Biomass as a Feedstock for Biofuel

Non-edible organic biomass, both terrestrial and marine, is often considered a viable feed stock for the production of next generation biofuels as such biomass is often underutilised and does not compete with existing human food supply chains (Milledge *et al.,* 2014). Algae and more specifically macroalgae have now more recently been considered a prospective biomass feedstock for biofuel production. Macroalgae's high growth rates compared to terrestrial plants as a result of physiological differences related to photosynthesis, whereby macroalgae is on average three to four times (7 vs 2%, respectively) more efficient when compared to most terrestrial plants is often cited as a distinct advantage for its use as a fuel. As such, macroalgae growth rates are reported to be in the range of 30-80 metric tons per hectare per year (MT/ha/year) when compared to that of other agricultural crops utilised in biofuel production such as corn, maize and sugarcane at 3–30 dry MT/ha/year (Milledge *et al.,* 2014). Furthermore, macroalgae cultivation does not require arable land, freshwater input or intense

management which represent major barriers for the economic production of terrestrial biofuel feedstock production.

The large diversity of macroalgae coupled with their high carbohydrate content implicates them as a relatively abundant source of renewable carbon that can be efficiently utilised for the production of net neutral carbon-based fuels and chemicals. Whilst highly diverse, it is large phaeophyceae that are considered to be the best option in terms of biofuel production as their high carbohydrate content and lack of lignin is particularly suited for fermentation derived products. However, many challenges still remain to be overcome before macroalgae biofuels can become economically viable.

Successful macroalgae derived biofuel production is dependent upon optimising processes of biofuel production at all stages of manufacture. Macroalgal biofuel manufacturing can be broadly categorised into four key areas:

i) Cultivation of macroalgae crop as feedstock
ii) Harvesting and transport of macroalgae crop
iii) Post-harvest treatment (cleaning, size reduction, preservation and storage etc)
iv) Energy extraction/fuel synthesis

As seen in the use of microalgae as a biofuel source, future success for macroalgae utilisation lies with improved methods of cultivation and harvesting for the economic viability of the industry. The seasonal nature of macroalgae growth is a particular challenge for commercial scale aquaculture/biofuel operations as it limits year-round production. Therefore, developments must be made in regard to storage/preservation, extending growth window or incorporating globalised supply chains in order to enable year-round fuel production in order to meet demand. As it stands these challenges represent major knowledge gaps for any emerging macroalgae biofuel industry (Black, 1955; Uchida & Miyoshi, 2013; Wout *et al.*, 2013).

Methods of production/extraction

Numerous methodologies exist for converting macroalgae biomass into a fuel source. Broadly speaking, these methods can be categorised into whether the biomass must be dried or can remain wet before production begins. The primary methods are outlined in the following table.

Table 2. Methods of energy extraction from macroalgal biomass (Milledge *et al.*, 2014).

Method	Utilises entire organic biomass	Requires biomass drying after harvesting	Primary energy product
Direct combustion	Yes	Yes	Heat
Pyrolysis	Yes	Yes	Primarily liquid by fast pyrolysis
Gasification	Yes	Yes [b] (conventional)	Primarily Gas
Biodiesel production	No	Yes [c]	Liquid
Hydrothermal treatments	Yes	No	Primarily Liquid
Bioethanol production	No [a]	No	Liquid
Biobutanol production	No [a]	No	Liquid
Anaerobic digestion	Yes	No	Gas

a) Polysaccharides require hydrolysis to fermentable sugars. Some of the sugars produced from the breakdown of seaweed polysaccharides are not readily fermented; b) Supercritical water gasification (SCWG) an alternative gasification technology can convert high moisture biomass; c) No current commercial process for the wet trans-esterification of wet macroalgal biomass.

Macroalgae have a water content of roughly 80-90%, higher than that of terrestrial crops (sugarcane ~75%, grain maize 14%–31%) (Rajkummar *et al.*, 2013; Zhou *et al.*, 2010; McLaren, 2009). The removal of water from macroalgal biomass is a highly energy intensive process but represents a critical step for many fuel production methods. As such, methods that require a dry feed stock are challenged from reduced energy return on investment. Air drying using solar energy is the most prevalent method due to its low cost but is restricted to certain climates and weather with the process taking on average 2-3 days. While solar drying remains the least expensive it is limited by volume that can be dried, with one square meter of area only capable of efficiently drying 100 grams of macroalgae material. Attempts have been made to utilise fossil fuels as means of driving evaporating but the burning of coal/oil etc to produce biofuel has generally proved to be uneconomical and counterproductive. The removal of water from macroalgae therefore requires advancements in efficiencies in regard to drying or alternatively developing and utilising methods that can tolerate wet feedstock are required in order to develop economically viable operations. Whilst technically challenging applications for fuels derived from macroalgae exists with the main methods of production outlined below.

Direct combustion

The simplest method of extracting energy from macroalgae is through burning its dried biomass. Historically, combustion has been used to burn dry biomass to provide heat for homes and to produce steam for electrical generation for use in industrial operations (Demirbas, 2001). Dried macroalgae is easy to ignite which is useful for initiating combustion but macroalgae's high carbohydrate content results in an overall low thermal output of 14–16 $MJ \cdot kg^{-1}$.

The use of *Ulva lactuca* as a dried solid fuel has been explored as a relatively simple method that avoids the numerous technological issues attributed to producing liquid fuels from macroalgae. *Ulva lactuca* has a growth rate of ~0.1-0.2 (in the literature) with a heating value of its dry biomass at 19 MJ/kg. Yantovski (2008), calculated that the energy output of dried *Ulva lactuca* through direct combustion is less than traditional photovoltaics however the initial energy input and costs to construct grow ponds is substantially less than solar systems providing and economic advantage. To produce a power output of 100kW, a pond surface area of approximately 4 hectares is required (Yantovski, 2008). Additionally, the ash bioproduct of algae combustion can be utilised as useful nutrient source to offset fertiliser input in the cultivation of macroalgae.

Pyrolysis

Pyrolysis is the thermal decomposition of materials at high temperature within an inert atmosphere (without air) to convert biomass to fuel, the same as that of the production of charcoal from wood (McKendry, 2002; Sairdur *et al.*, 2011). Pyrolysis consists of heating biomass above its decomposition temperature acting to break chemical bonds and simplify compounds. Pyrolysis can output high volumes of fuel relative to the initial feed biomass and the method of production can be optimised to produce bio-oil, syngas or char depending on the desired fuel.

Song *et al.*, (2014) evaluated pyrolysis (combined with solvents) to produce a renewable energy source in the form of bio-oil utilising *Enteromorpha prolifera* as a feedstock. Their findings suggest that thermal cracking is an effective method to produce bio-oil from *Enteromorpha prolifera* and has the ability to be easily applied to exiting thermal cracking infrastructure. The

studied produced a maximum bio-oil yield of *Enteromorpha prolifera* using vacuum gas oil as a solvent to be 90.5% at 300 °C with a reaction period of 30 minutes. Song *et al.*, (2014), identifies bio-oil produced from *Enteromorpha prolifera* through pyrolysis has the potential to be produced on an industrial scale.

Gasification

The gasification process converts organic biomass material at high temperatures into a combustible gas (syngas) that has a calorific 4–6 MJ/m^{-3} (McKendry, 2002). Syngas is comprised of a mixture of gases including hydrogen (30-40%), carbon monoxide (20-30%), methane (10%–15%) and others in small quantities. This gas mixture can be combusted to generate heat for the production of in gas turbine systems (Demirbas, 2001; McKendry, 2002). Additionally, syngas gasification can be used to produce methanol and hydrogen as a fuel for transport as well as other uses however producing fuel in this manner is estimated to cost 1.5 to 4 times as much as the same fuel produced using fossil fuels.

A review by Brennan & Owende (2010) examined the gasification process of algae with a focus on the energy balances involved as well as the underlying methodology. Whilst data on macroalgae gasification is limited, they concluded that if gasification can be achieved using wet biomass it would be more economically and energetically viable as a fuel source. Such an improvement would allow for a more competitive process, one that would compete with anaerobic processes as higher yields combined with gasification being a far more rapid process would demonstrate clear operational benefits.

Liquefication - Biodiesel production

The hydrothermal liquefication process utilises low temperatures but with high pressures to convert organic biomass in the presences of catalyst and hydrogen into a stable liquid hydrocarbon fuel (Demirbas, 2001). Hydrothermal liquification can be considered analogous to that of the production of char via pyrolysis but for that of a liquid biofuel. In general, the complex methodology associated with producing fuel via the liquidation process has been viewed as uneconomical due to the multifaceted feed systems and higher costs than that of gasification or pyrolysis (McKendry, 2002).

The production of bioethanol derived from terrestrial crops such as corn and sugarcane for the use as a renewable fuel source is now a well-established industry (Yang *et al.*, 2011). However, to meet ever increasing energy demands, ethanol derived from cellulosic biomass was expected to be utilised to meet these demands (Ferrell & Sarisky-Reed, 2010). Nonetheless, despite extensive research and the large availability of low-cost lignocellulosic material such as straw, no large-scale commercial production of biofuel from this material source currently exists (Balat & Balat, 2008). A major barrier for the production of bioethanol from straw and other second-generation materials is that of lignin, which is commonly found in many terrestrial plants and inhibits the degradation of cellulose. As such, macroalgae have been proposed as a viable feedstock for bioethanol production as they typically contain low quantities of lignin (Yanagisawa, 2013).

Brown, green and red algae have all been investigated and utilised as a feedstock for bioethanol production, however brown algae, with its high carbohydrate content and ability to be cultivated on mass has been suggested as the most promising for commercial operations (Jung *et al.*, 2013). *Laminaria hyperborean* contains relatively high levels of laminarin and mannitol which have been found to yield high levels of ethanol when fermented with yeast (Huesemann *et al.*, 2012). On average, macroalgae typically yield between 0.08 and 0.12 $kg \cdot kg^{-1}$ dry seaweed depending on both the species and method of production, but Wargacki *et al.*, (2012) found higher yields to be possible through experimental methods with up to 80% of the theoretical maximum achieved at 0.296 g ethanol g^{-1} dry seaweed. Furthermore, Aizawa *et al.*, (2007) estimated commercial operations could effectively yield 0.296 g ethanol g^{-1} dry seaweed.

Anaerobic digestion

The anaerobic digestion of macroalgae by bacteria has been demonstrated to be a viable method for the production of biogas as an energy source (Milledge *et al.*, 2014). One recent example is that of Tokyo gas, which demonstrated that 20 m^3 of methane can be produced from one tonne of macroalgae and was used to power 9.8 kW electrical generation plant (Huesemann *et al.*, 2010). One estimate places biogas produced from macroalgae derived from anaerobic

processes could offset greenhouse gas emissions from natural gas by between 42%-82% (Florentinus *et al.*, 2008).

The bacteria involved in the production of methane through anaerobic digestion are highly sensitive to the chemical composition of the growth feedstock. Methane yield is therefore determined by the content of carbohydrates, proteins and lipids of the digested substrate (Park & Li, 2012). It has been suggested that the chemical composition of macroalgae make them especially suitable for the production of biogas (Roesijadi *et al.*, 2010).

Macroalgal Biomass as a Fish Feed

Currently, there exists a great demand for alternative sources of aquaculture feed. Commercially formulated aquaculture feed accounts for a significant percentage of finfish aquaculture production costs typically in the range of 50-80% of an operations total budget. Moreover, a continued rising demand for finfish globally coupled with a stagnation in the production of fish meal and fish oil feed products derived from wild fish stocks has led to an increase in feed stocks costs (Shepherd & Jackson 2013). As a result, there is a large push from both commercial operations as well as research institutions in developing alternative feed stocks to replace or offset fish-based feeds. Ultimately, for a successful feed replacement to occur either wholly or as a partial ingredient offset, growth rates must be maintained. Furthermore, a replacement should not affect the final product quality, the overall health of the fish and should limit environmental impacts. In recent years macroalgae have been suggested as a viable substitute for fish-based protein in cultured fish feed.

Macroalgae's high essential amino acid and high protein content combined with its trace metal and vitamin composition position it as a potential low-cost source of protein. Nakagawa *et al.*, (1984) is one of the earliest studies to explore the use of macroalgae inclusion into finfish diet. The study utilised dried and milled green algae, *Ulva pertusa* as a substitute for fishmeal in the diet of black sea bream (*Acanthopagrus schlegeli*). Their research demonstrated that the substitution of as little as 10% macroalgae into the fishes' diet produced elevated protein efficiency, with other growth measurements being unaffected. This research has spurred further investigations into the application of macroalgae as a fish feed additive, investigating both a

range of macroalgae and fish species. Despite this, when compared to the number of macroalgae species known to science, few have been fully evaluated as a potential feed and research is typically limited to studying the diet of key carnivorous fish species of high commercial value such as trout, salmon, seabream, seabass and flounder (Wan *et al.*, 2018).

Wan *et al.*, (2018) conducted an extensive literature review of studies investigating the effect that the inclusion of macroalgae in the diet of finfish has upon their growth performance. The data of which is presented below.

Table 3. The effects of dietary macroalgae inclusion has on farmed finfish growth performance.

Algae	Fish	Natural diet†	Inclusion (%)	Duration (weeks)	Growth effect	References
Chlorophyta						
Ulva (Enteromorpha) intestinalis	Nile tilapia (fry)	H	10, 20, 30, 40, 50	6	↓ FW, WG, SGR, & Total Fl when inclusion ↑ ↔ Feed consumption, FCR, & PER	Thi *et al.* (2015)
Ulva intestinalis (Protein concentrate)	Nile tilapia (fry)	H	3.9, 7.8, 11.7	12.9 (90 days)	↓ WG, SGR, & Fl at 7.8 & 11.7% ↓ Fl at 3.9% ↔ WG & SGR at 3.9% ↔ FCR & protein retention ↓ Lipid retention	Serrano and Aquino (2014)
Ulva lactuca	Gilthead seabream	O	2.6, 7.8 (exp 1) 14.6, 29.1 (exp 2)	15.9 (exp 1) 20.1 (exp 2)	↔ No change (exp 1) ↓ FW & SGR at 29.1% (exp 2) ↔ FW, SGR, WG at 15.9% (exp 2)	Shpigel *et al.* (2017)
Ulva lactuca	European seabass	C	5, 10, 15	8	↑ FW, WG, protein productive value but ↓, when % inclusion ↑ FCR when % inclusion ↑ ↔ Fl & PER	Wassef *et al.* (2013)
Ulva lactuca	African catfish	C/O	10, 20, 30	10	↔ FW, BL, WG, CF, SGR, FCR, PER at 10% inclusion ↓ Fl, daily feed take & protein productive values at 10% inclusion ↓ FW, BL, WG, CF, SGR, Fl, Daily Fl, & PER at 20 & 30% inclusion ↑ FCR at 20 & 30% inclusion	Abdel-warith *et al.* (2015)
Ulva lactuca	Striped mullet	O/D	10, 15, 20, 25	15	↑ FW, WG, SGR, Percent WG, PER when % inclusion ↑ ↑ WG from 20% ↓ FCR when % inclusion ↑	Wassef *et al.* (2001)
Ulva lactuca	Rainbow trout	C	10	8.6 (80 days)	↓ WG, Relative growth rate, & SGR	Yildirim *et al.* (2009)
Ulva linza	Rainbow trout	C	10	8.6 (80 days)	↓ WG, Relative growth rate, & SGR	Yildirim *et al.* (2009)
Ulva rigida	Nile tilapia	H	10, 20, 30	10.7 (75 days)	↓ FW; Relative growth rate, daily WG & PER at 30% ↑ FCR at 30%	Azaza *et al.* (2008)
Chlorophyta						
Ulva rigida	Common Carp	0	5, 10, 15, 20	16	↔ FW, WG, FCR, SGR, PER at 5–15% ↑ Fl at 5%, FCR at 20%, & ANEU at 5–15% ↓ All growth parameters at 20%	Diler *et al.* (2007)
Ulva rigida	Nile tilapia	H	Lo lipid + 5% Hi lipid + 5%	16	↑ FW, SGR, PER & net protein utilisation ↑ Net energy utilisation with Hi *Ulva* ↓ FCR	Ergün *et al.* (2008)

Ulva rigida	Gilthead seabream	O	5 *Ulva* +13, 16, 19, 22 lipid	7	↔ FW, SGR, FCR, PER, & NPU at 13, 16 & 19% lipid +5% *Ulva* ↑ FW, SGR, & PER at 22% lipid +5% *Ulva* ↓ FCR at 22% lipid +5% *Ulva* ↔ NPU at 22% lipid +5% *Ulva*	Emre *et al.* (2013)
Ulva rigida	Rainbow trout	C	5, 10	12 (+3 starvation)	↓ Weight loss after starvation	Güroy *et al.* (2011)
Ulva rigida	European seabass	C	5, 10	10	↔ PER, VFI ↓ FW, Daily WG when % inclusion ↑ ↑ FCR when % inclusion ↑	Valente *et al.* (2006)
Ulva rigida	Gilthead seabream	O	5, 15, 25	10	↔ FW & SGR at 5 & 15% ↔ CF & FCR ↑ FW & SGR at 25%	Vizcaíno *et al.* (2015)
Ulva rigida	Nile tilapia	H	5, 10, 15	12	↓ FW, WG, SGR, & ANEU at 15% ↑ FCR, Dietary protein & energy utilised at 15% ↔ FI & ANPU	Kut Guroy *et al.* (2007)
Ulva spp.	Nile tilapia	H	10	12	↔ FW, SGR, FI, FCR, & PER	Silva *et al.* (2014)
Ulva spp. *(1:1, U. rigida:U. lactuca)*	Nile tilapia	H	10, 15, 20	9	↓ FW, SGR at 15 & 20% ↑ FCR when % inclusion ↑ ↑ PER at 10 & 15%, ↔ PER @ 20%	Marinho *et al.* (2013)
Ulva pertusa	Black seabream	O	10	20.4 (143 days)	↔ Growth rate, FE, ↑ PER ↓ FW, growth rate	Nakagawa *et al.* (1984)
Chlorophyta						
Ulva ohno (derived meal)	Atlantic salmon	C	2.5, 5%	12	↔ FW, FI, WG, FCR, SGR, PER, protein growth rate, net protein cultivation & CF.	Norambuena *et al.* (2015)
Ulva (Enteromorpha) prolifera	Large yellow croaker	C	5, 10, 15	10	↑ FW, when % inclusion ↑ ↓ Protein Retention when % inclusion ↑ ↔ Feed efficiency ratio, feeding rate	Asino *et al.* (2011)
Rhodophyta						
Eucheuma denticulatum	Asian seabass	C	5	8	↔ FW, WG, SGR, Total FI, FCR, PER, NPU, & CF	Shapawi and Zamry (2016)
Eucheuma denticulatum	Japanese flounder	C	3, 6, 9	8	↔ FW, WG, SGR, & PER at 3% ↓ FW, WG, SGR, & FER at 6 & 9% ↔ FI	Ragaza *et al.* (2015)
Gracilaria bursa-pastoris	European seabass	C	5, 10	10	↔ FW, Daily WG, FCR, PER, VFI	Valente *et al.* (2006)
Gracilaria cornea	European seabass	C	5, 10	10	↔ PER & VFI ↓ FW &WG when % inclusion ↑ ↑ FCR when % inclusion ↑	Valente *et al.* (2006)
Gracilaria cornea	Gilthead seabream	O	5, 15, 25	10	↔ FW, SGR, FCR, & CF at 5 & 15% ↔ FW & CF at 25% ↓ SGR at 25% ↑ FCR at 25%	Vizcaíno et al. (2015)
Gracilaria lemaneiformis	Black seabream	O	5, 10, 15	8	↔ FW, WG, & feed efficiency ratio at 5, 10, 15 ↓ FW, WG, & feed efficiency ratio at 20%	Xuan *et al.* (2013)
Rhodophyta						
Gracilaria lemaneiformis	Rabbit fish	O	33	8	↓ FW, WGR, SGR, PER ↑ FCR	Xu *et al.* (2011)
Gracilaria vermiculophylla	Nile tilapia	H	10	12	↓ FW, SGR, FI, & PER ↑ FCR	Silva *et al.* (2014)
Gracilaria vermiculophylla	Rainbow trout	C	5, 10	13	↔ FW, SGR, FCR, & VFI at 5% ↓ FW, SGR, & VFI at 10% ↑ FCR at 10%	Valente *et al.* (2015a, 2015b)
Gracilaria vermiculophylla	Rainbow trout	C	5, 10	13	↔ BL, Daily growth index, PER, & FCR at 5% ↓ FW, BL, Daily growth index, & PER at 10% ↑ FCR at 10%	Araújo *et al.* (2016)
Gracilaria pygmaea	Rainbow trout	C	3, 6, 9, 12	7	↑ FW, at 6 & 9% ↓ SGR at 12% ↓ FCR at 3, 6 & 9% ↑ FI as ↑ inclusion % level	Sotoudeh and Jafari (2017)
Kappaphycus alvarezii	Asian seabass	C	5	8	↔ FW, WG, SGR, Total FI, FCR, PER, NPU, & CF.	Shapawi and Zamry (2016)
Kappaphycus alvarezii	Asian seabass	C	6 (raw) & 6, 10, 14, 18, 22 (cooked)	10	↔ FW, WG, SGR, Total FI, Daily FI, FCR, PER, NPU. & CF at 6% Raw ↑ FW, WG, SGR, PER, NPU. & CF, & ↓ FCR at 6% cooked ↓ FW, WG, SGR, PER, NPU. & CF, & ↑ FCR at % cooked ↑ (*generally*)	Shapawi *et al.* (2015)
Palmaria Palmata	Atlantic salmon	C	5, 10, 15	14	↔ FW, WG, CF, FCR, & SGR	Wan *et al.* (2016)
Porphyra dioica	Nile tilapia	H	10	12	↔ FW, SGR, FI, FCR, & PER	Silva *et al.* (2014)

Porphyra dioica	Rainbow trout	C	5, 10, 15	12.5	↑ FW at 15% ↔ WG, SGR, FCR, FE, PER, protein retention efficiency, & VFI.	Soler-Vila *et al.* (2009)
Porphyra purpurea	Grey mullet	O	16.5, 33	10	↓ FW, WG, SGR, Daily FI, FE, PER, & Net Protein Utilisation when % inclusion ↑ ↑ FCR when % inclusion ↑	Davies *et al.* (1997)
Porphyra spp.	Atlantic cod	O	5.5, 11	12	↔ FW, FI, Percentage growth, SGR, & FCR.	Walker *et al.* (2009)
Pterocladia capillacea	European seabass (fry)	C	5, 10, 15	8	↑ FW, WG at 5% ↓ FW, WG at 10 & 15% ↑ FCR at 15% ↓ PER at 10 & 15% ↓ PPV at 15%	Wassef *et al.* (2013)
Pyropia (Porphyra) yezoensis (spheroplasts)	Red seabream	C	5	6	↑ FW, WG, % Growth, SGR, PRR, LRR, & PER ↓ FCR	Kalla *et al.* (2008)
Pyropia (Porphyra) yezoensis Ueda	Nile tilapia	H	15, 30	10	↑ FW, WG, SGR, FI, PER, PPV, & ALC at 15%	Stadtlander *et al.* (2013)
Pyropia yezoensis (autoclave extract)	Olive flounder	O	0.5, 1, 1.5, 2	9	↔ FW, WG, Daily growth rate, & Daily FI at 5, 10, 15% ↔ Feed efficiency at 5 & 10% ↑ Feed efficiency at 15% ↔ FW & Daily FI at 20% ↓ WG, daily growth rate, & feed efficiency at 20%	Choi *et al.* (2015)
Phaeophyceae						
Ascophyllum nodosum	Red seabream	C	2.5, 5	7.1 (50 days)	↓ WG, Feed conservation efficiency, ↓ PER & BL at 2.5% ↑ BL & FW at 5%	Nakagawa (1997)

Algae	**Fish**	**Dietary group**	**Inclusion (%)**	**Duration (weeks)**	**Growth effect**	**References**
Phaeophyceae						
Ascophyllum nodosum	Red seabream	C	5, 10	10	↓ FW at 10% ↓ FE at 10% ↑ FE at 5%	Yone *et al.* (1986)
Cystoseira barbata	Nile tilapia	H	5, 10, 15	12	↑ Dietary protein & energy utilisation at 15% ↔ FW, WG, SGR, ANEU, & FCR	Kut Guroy *et al.* (2007)
Ecklonia cava	Olive flounder	C	6	6	↔ FW, WG, FCR, PER, FI, & CF	Kim *et al.* (2014)
Macrocystis pyrifera	Rainbow trout	C	15, 3, 6	17.7 (124 days)	↓ FW & SGR when % ↑ ↔ FCR	Dantagnan *et al.* (2009)
Sargassum polycystum	Asian seabass	C	5	8	↔ FW, WG, SGR, FCR, PER, NPU, & CF ↑ FI	Shapawi and Zamry (2016)
Sargassum fusiforme	Olive flounder	C	6	6	↔ FW, WG, FCR, PER, FI, & CF	Kim *et al.* (2014)
Undaria penatifida	Red seabream	C	5, 10	10	↑ FW at 5% ↑ FE	Yone *et al.* (1986)

↑, increase; ↔, no change, ↓, decrease compared to the control diet ($P < 0.05$). C, carnivore; O, omnivore; H, herbivore; D, detritivore; ANEU, apparent net energy utilisation; ANPU, apparent net protein utilisation; BL, body length; CF, condition factor; FCR, feed conversion ratio; FE, feed efficiency; FI, feed intake; SGR, specific growth rate; FW, final weight; PER, protein efficiency ratio; WG, weight gain; VFI, voluntary feed intake Wan *et al.*, (2018).

This data demonstrates that the addition of macroalgae to cultivated finfish feed is a viable solution in helping to mitigate global fish stock decline and offers up attractive economic opportunities for macroalgal biomass whether produced for purpose or as a by-product.

The demand for macroalgae is increasing not only as a food source but also as a reservoir for the bioactive chemical compound and the material biomass they provide. With pharmacological, nutraceutical, cosmetic, fuel synthesis and feedstock applications, macroalgae represent a source of major economic potential. However, as demands grows and coastal ecosystems continue to be degraded worldwide, the impetus to harvest more macroalgae grows. Consequently, the low (relative to agriculture) quantity of cultivated macroalgae presents a major threat of overharvesting for wild populations as a consequence of commercial overexploitation. Such exploitation would not only effect localised macroalgae populations but has the potential to result in biodiversity loss for a large number of species and would act to degrade the overall environment. As such, the need arises to expand aquaculture operations both in terms of inland infrastructure as well nearshore and offshore sites.

Macroalgae produce a vast array of chemically unique bioactive compounds. However, for most industries, commercial operations require only one or a limited number of these compounds for their manufacturing processes and products. Therefore, there is a strong need to advance our knowledge of how macroalgae metabolisms and bioactive chemical formations works as to develop cultivation and extraction methods that better optimise this process. Whilst the research goals are clear, many challenges remain.

As it stands, the cultivation of macroalgae in an economically viable and productive manner is presented with serious challenges. While a long history of macroalgae research exists, the vast diversity of groups and species combined with their physiological complexity means fundamental research is still required in order to develop basic knowledge on things such as primary growth factors. Such work is crucial before any successful commercial operation can take place. Furthermore, significant work remains in terms of optimising established aquaculture operations. More specifically, progress needs to be made in terms of disease resistance, the identification of fast-growing species and the determination of factors that control the synthesis of desired compounds. Additionally, new technologies that improve aquaculture system designs as to be more robust and efficient and make use of algal biomass as a fuel and feedstock source are crucial for developing a more sustainable economies in the future.

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