Research proposal

A. PROJECT TITLE	**Preventing sun-induced skin damage with New Zealand algae-derived bioactives**
"SHORT" TITLE	Seaweed sun defence
B. THEME / PROGRAMME	Blue Economy Innovation Fund

C. PROJECT KEY RESEARCHERS

Role	Name	Institution / company	Email
Project Leader	Mike Packer	Cawthon Institute	mike.packer@cawthron.org.nz
Project Co-Leader	Tom Wheeler	Cawthron Institute	tom.wheeler@cawthron.org.nz
	Gary Fisher	University of Michigan	
	Andy Elliott	Wakatū Incorporation	
	Paul South	Cawthron Institute	
	Jonathan Puddick	Cawthron Institute	
	Jonathan Banks	Cawthron Institute	

D. PROJECT PARTNERS

Name	Organisation / company / agency / Iwi / Māori	Role in project
Wakatū Incorporation	Company	Seaweed biodiversity study on marine farms, supply of material, commercial development.
SRW Laboratories Ltd	Company	Market insight, formulation trials of early candidate extracts, commercial development.

E. ABSTRACT/SUMMARY

Current sun protection products aren't fully-effective, have damaging side-effects and negative environmental impacts. With increasing awareness of the harm caused by sun-damage, the sunburn prevention market is projected to grow by over NZ$4B globally by 2022[1]. There is an opportunity to develop innovative products to fulfil this demand and create opportunities for Aotearoa's blue economy. **Algae have evolved finely-tuned ultraviolet radiation (UVR) control and damage-mitigation processes to ensure their survival**, and therefore they provide a 'natural' source for sun-protective compounds. This project couples the light management strategies *and* immunomodulatory bioactives produced in algae, to deliver an entirely new class of suncare proof-of-concepts to prevent and ameliorate human sunburn injury.

This builds on knowledge that **processes underlying sunburn are immunological responses to UVR irritation**, and the demonstration that immunomodulatory activity of algal bioactives can be employed as active protection for beneficial effect during the sunburn reaction.

Identifying endemic and native species to commercialise will provide new options for seaweed farming to create a high-value sustainable blue economy industry in Aotearoa. This project will lead to deeper understanding of our algal biota and their chemical potential. It will assess NZ algal species for UVR-absorbing and sunburn immune response-modulating compounds. The UVR absorbers can be used in sunscreen products as alternatives to currently-available molecules that have a range of associated issues. The immunomodulatory bioactivities we will assess, beneficially modulate the signalling pathways driving the skin's response to sun damage, especially oxidative damage-induced changes in the immune response of skin to UVR.

F. PROBLEM DEFINITION/OPPORTUNITY

Agents used to block or absorb UVR are not 100% effective, making cumulative skin damage inevitable. Many sunscreens have damaging side-effects and are increasingly being banned due to their environmental impact[2, 3]. Consumers need new approaches and better products that don't harm the environment.

The cost of acute sunburn treatment alone is in the billions of dollars per annum excluding indirect costs for treatment of skin cancer and skin-aging resulting from sunburn and UV damage[4-7], with NZ and Australia having the highest rates of skin cancer in the world[8]. The opportunity for NZ is to create a step-change in a large market currently based on products aimed only at prevention. This programme will enable next-generation products that offer more efficacious prevention of sun-damage with fewer side-effects, but also create innovative products that combine defence with amelioration of sun-induced damage.

Currently the sunburn skincare category is based on protection during UVR exposure. Sunscreens, make-ups and lip-care products contain UV filters to protect users. Today's protective agents have significant shortfalls, including safety risks[9, 10]. As consumers demand higher sun protection factors (SPFs) they are exposed to higher levels of the problematic agents.

Inorganic (physical) UV blockers in sunscreens include zinc and titanium oxides to reflect or scatter broad spectrum light. There are concerns that penetration of the blockers results in increased cytotoxic and genotoxic reactive oxygen species (ROS) in the skin by photoactivation[9, 11]. Modern formulations contain nano-sized particles to avoid opaqueness and to make them easier to apply, but this increases the penetration of the blockers and increases health concerns. The impact of these nanoparticles on the natural environment is also a major issue as they can be ecotoxic[12-16].

Organic light-absorbing compounds used in sunscreens are also problematic. The most widely used are allergens, causing contact dermatitis[10, 17-21]. Others accumulate in human tissues disrupting endocrine levels[22, 23], affecting thyroid levels and interrupting reproductive- and neurological-development[24, 25]. Some are indicated as carcinogenic[22, 26].

Many of the UV filters also have ecotoxicological effects. It is estimated that up to 25% of the sunscreens we apply accumulate in the aquatic environment[27]. Commonly used sunscreens are being banned because of their deleterious effect on corals and other marine life[2, 3, 15, 28].

The opportunity in this project, is to leverage the natural UVR-protection strategies of algae and other compounds in them for new sunscreen concepts and next-generation sun protection products. These will not only reduce sunburn but also enhance treatment for sunburn, using environmentally-friendly and sustainable approaches based on our knowledge of the sun mammalian UVR response through prevention of oxidative damage[29-32] (Fig. 1).

There are amelioration opportunities presenting well after UVR exposure, in contrast to current approaches of only blocking UVR. Our new approach is to combine passive and bioactive UVR control to target the range of mechanisms acting during sunburn. These approaches will form the basis of new high-value export products, favoured by consumers because they lead to better health and environmental outcomes.

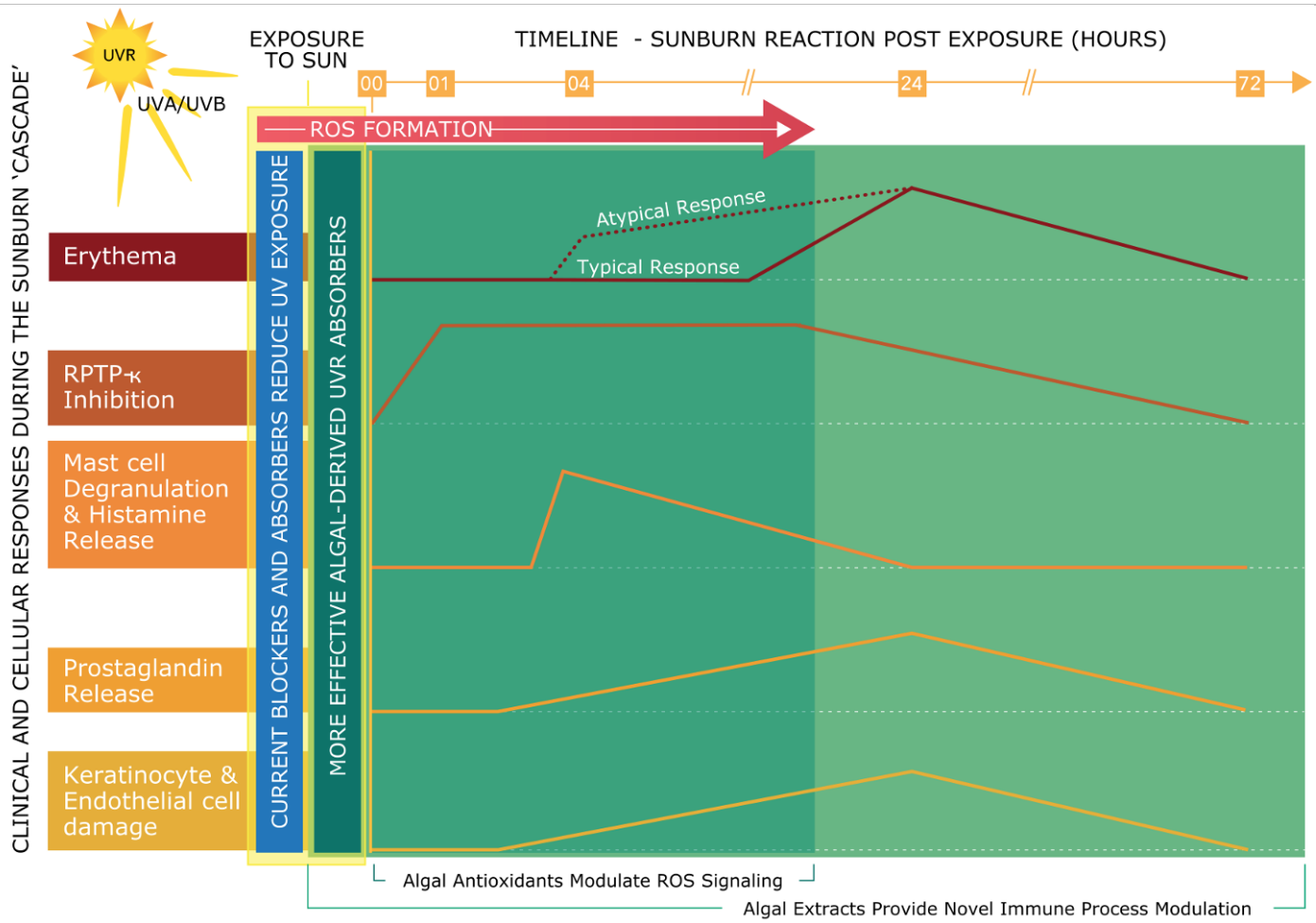


Figure 1. A selection of processes occurring during sunburn and the window of benefit opportunity. Current approaches target only the UVR exposure window (**blue**). This project (**green**) introduces UVR control with new algal bioactive derivatives to complement algal-derived photosynthetic UVR absorbers. Our further innovation is to modulate events during and post-exposure for beneficial outcomes. RPTP-κ = receptor-type protein-tyrosine phosphatase kappa. ROS = reactive oxygen species.

G. OUTPUT/SOLUTION

Some algal compounds that function as built-in sun protection[33-37] are beginning to be used overseas in high-value suncare products as 'natural sunscreens', but we have no knowledge of the potential of NZ algae as a resource for this purpose. In addition to these UVR absorbers, we seek targeted antioxidants that modulate *specific* ROS effects and the immune signalling occurring during mammalian UVR-response, to improve post-sunburn outcomes[29-32, 38].

Outputs:

1. Establish the diversity and abundance of seaweed species on marine farms at either end of the South Island.
2. Determine the abundance of compounds in a range of algal species with UVR absorption and immunomodulatory activities useful for our new concept suncare products:
    - Species selection will target Aotearoa's endemic and native species and be informed by marine farm presence and end-user partners input.
3. Develop methods of assessing and preparing extracts enriched for these compounds:
    - Develop a new scalable bioassay to allow large numbers of samples to be investigated for the properties required
    - Methods of preparing extracts selected will be informed by practical ability to scale production to commercial application by stakeholders.
4. Determine potential of specific algal species as a source of ingredients for next-generation suncare products:
    - Each species with useful compounds that can be extracted with high enough purity will be assessed for production is commercially-, environmentally- and culturally-viable by involving end-users

- The IP position of each candidate will be evaluated in the context of mātauranga Māori and WAI262.

H. PROPOSED RESEARCH/APPROACH

New approaches to prevent and mitigate sunburn damage are needed. We have a multi-layered and iterative approach to detect the compounds of interest in NZ algal species for use in environmentally-friendly and innovative suncare products. Compounds will be derived from endemic and native seaweed species provided by our partner Wakatū Incorporation as part of a biodiversity study on marine farms and from promising algae in the Cawthron Institute Culture Collection of (CICCM).

Following taxonomic identification of the seaweeds (detailed below), we will select candidates for chemical analysis based on:

- Species naturally growing on marine farming systems that are presently treated as waste; which could provide additional or new revenue streams, alleviating pressure to expand operations and occupy more space.
- Species that can already be grown on commercial-scale by the aquaculture industry.
- Endemic and native seaweeds to unlock rawa taiao (natural resources) allowing NZ to leverage our unique biodiversity whilst also alleviating the ecological risks associated with cultivating introduced seaweed species.
- Red seaweeds and cyanobacteria (from the CICCM) that are known to produce mycosporine-like amino acids (MAAs), UVR-absorbers that are emerging in environmentally-friendly and coral-friendly high-value sunscreens.
- Additional species will be included based on literature information of their potential[9, 36, 39, 40].

Wakatū Incorporation has access to marine farms located from the bottom to the top of the South Island. The geographical variation is important as the different temperatures along the length of the South Island may affect the seaweed species' potential for containing the kinds of bioactive compounds we target. This large geographical spread will not only affect diversity but also abundance of algae. It is not yet known that such farms contain a consistent suite of species across them – this is an important consideration and the observations made will be an outcome of the work. Wakatū Incorporation will supply seaweed material from these farms for taxonomic identification and we have designed our sampling regime so that we can fractionate and analyse the material chemically.

To provide seaweed material for the chemical analysis and obtain a firm understanding of its availability, we will quantify the seasonal abundance of macroalgal biofouling at a range of spatial scales such as between geographic regions, areas of a farm and depths.

We will begin sampling in the autumn of 2021, collecting five 1 m samples of all of the algal material from each spatial scale combination on a seasonal (every 3 months) basis for 18 months. The samples will be identified to lowest possible taxonomic resolution using morphological characteristics with voucher specimens collected to ensure consistency across the programme. The data will be used to assess patterns of algal biodiversity on marine farms and describe the relative abundance of algal species. Based on our extensive experience sampling algal fouling on farms and rocky reefs, we expect to encounter many species of red, brown and green seaweeds. As abundance will vary according to season, there will be a trade-off between the amount of material needed for chemical analysis and availability, so if one species looks promising but is in low abundance, we will modify our sampling regime to compensate. This is also the reason for sampling over more than a single annual cycle. The taxonomy and nomenclature for some of these species can be complex, therefore where possible we will further employ molecular techniques to determine their identity with more certainty.

Two approaches are available for molecular species identification, metabarcoding and PCR/sequencing. Both are in current use at Cawthron. The "barcoding" regions of the cytochrome c oxidase subunit 1 (COI) and the ribulose bisphosphate carboxylase large subunit gene (*rbc*L) gene will be used to distinguish species of red (Rhodophyta[42]), brown (Phaeophyceae) and green (Chlorophyta) algae. There are sequences for more than 5,000-species of red algae and 5,000 species of brown algae listed on the Bar Code of Life database and 2,000+ species of green algae[43, 44].

Robust DNA extraction methods for red and brown algae are available and primers suitable for sequencing this barcoding region from a wide range of genera have been published. Morphological characters and molecular barcodes will be used to ensure the integrity of feedstock for the chemical extractions.

Samples from the collected material will then undergo a first-pass chemical analysis. Subsamples of seaweed will be extracted with water and methanol for initial biochemical assessment. This will allow a wide range of seaweed samples to be screened for MAAs and to reveal the diversity of agents that prevent oxidative epidermal growth factor receptor (EGFR) activation in our new cell-based bioassay described below.

Red seaweed species and cyanobacteria are a known sources of UVR-absorbing MAAs. We will search for these in the methanol extracts of algae using established high-performance liquid chromatography (HPLC, reverse phase C8 column) together with commercially available standards (porphyra-334, shinorine, mycosporine-glycine, mycosporine-2 glycine, mycosporine-taurine, asterina-330, palythene, palythine, and palythinol).

Beyond UVR absorbers like MAAs, the ultimate goal coming out of our approach is to identify compounds in algal extracts that can protect receptor-type protein-tyrosine phosphatases (RPTPs) from UVR-induced oxidative damage as they are particularly sensitive. We have a patented biochemical assay where immunoprecipitation using an antibody specific to the oxidised active site of the phosphatase will allow us to determine the ratio of oxidised RPTP-κ to total RPTP-κ using western blot analysis[38]. We have used this previously to show a methanol extract of a non-NZ brown seaweed contains a compound that can protect RPTP from the oxidative damage that activates it in the mammalian UVR response[38]. The tool allows us to determine effectors that potentially offer specific protection for RPTP therefore modulating this ROS effect and is therefore novel as current antioxidant therapies quell *all* ROS activity, making them unhelpful or even detrimental by suppressing both beneficial and harmful effects.

The main aim of this project is to develop a new cell-based bioassay that will allow for greater number of samples to be investigated for this property based on our knowledge of the pathway and sequence of events during sunburn (Fig. 1)[29-32]. The bioassay determines EGFR activation using in mammalian cell lines that are exposed to oxidising hydrogen peroxide in culture. As the coordinator of the mammalian UVR response, EGFR activation is a downstream event to RPTP oxidation, where oxidised inactivated RPTP results in higher levels of activated EGFR. We have developed a proof-of-concept of this new assay using transient expression of both EGFR and RPTP in Chinese Hampster Ovary (CHO) cells in culture[30, 38] utilising commercially-available ELISAs. This works well and could be used as is, but we will look for commercially-available cell lines that constitutively express either or both of these proteins as well or we will make stably-transfected lines, and will explore other methods assessing EGFR activation to result in a faster assay with consistent outputs that is more suitable for screening and bioassay-directed fractionation purposes.

The crude extracts that are active in the bioassays for modulating sunburn damage pathways above, will then be further investigated using bioassay-directed fractionation. Seaweed specimens will be extracted using different solvents and undergo liquid-liquid phase-partitioning to assess the solubility of the active component/s. These fractions will be assessed in the oxidative EGFR activation assay and some samples further analysed for the oxidation status of RPTP-κ, providing valuable information for scale-up extraction options and formulation. The data will provide information on the identity of the active component/s, guidance on pathways towards identification of the active/s (to be conducted in subsequent projects) and information on whether different types of seaweed display similar RPTP protection and seasonal variations in its concentration.

These research findings will be fed to our industry partners continuously throughout the project to develop proof-of-concept formulations and products containing candidate algal extracts of promising leads.

Critical Skills and Team

Disruptions occur when diverse technologies converge, and this team consists of the right mixture of skills to deliver the research proposed and ensure effective commercial outcomes for end-users. The team is led by Dr Mike Packer who previously led a MBIE-funded programmes (Healthy and Functional Food Ingredients from NZ Algae and Greenshell™ Mussel) that provided foundational knowledge on immunomodulatory effects of algal extracts for this proposal[44-47] and also (Good Enough to Eat) on other aspects of algal biotechnology. His relevant algal experience

includes biotechnology, cell culture, bioactives, bioassays, and potential health products [48, 49] and he is well versed in the biochemical methods required (including immunoprecipitation, ELISA and Western blot analysis, transfection and expression of exogenous proteins in mammalian cells lines).

Complementary foundational knowledge comes from Gary Fisher's University of Michigan laboratory, bringing unique expertise in clinical and molecular dermatology. This laboratory's insights into molecular mechanisms of sunburn together with patented methods for discovering selective antioxidants for RPTP-κ inform and enable this research[30, 38, 50, 51]. Furthermore, Fisher's experience with UVR skin bioassays and clinical trials for sunburn is critical for establishing safe and credible trials for the products developed from this project [50, 52].

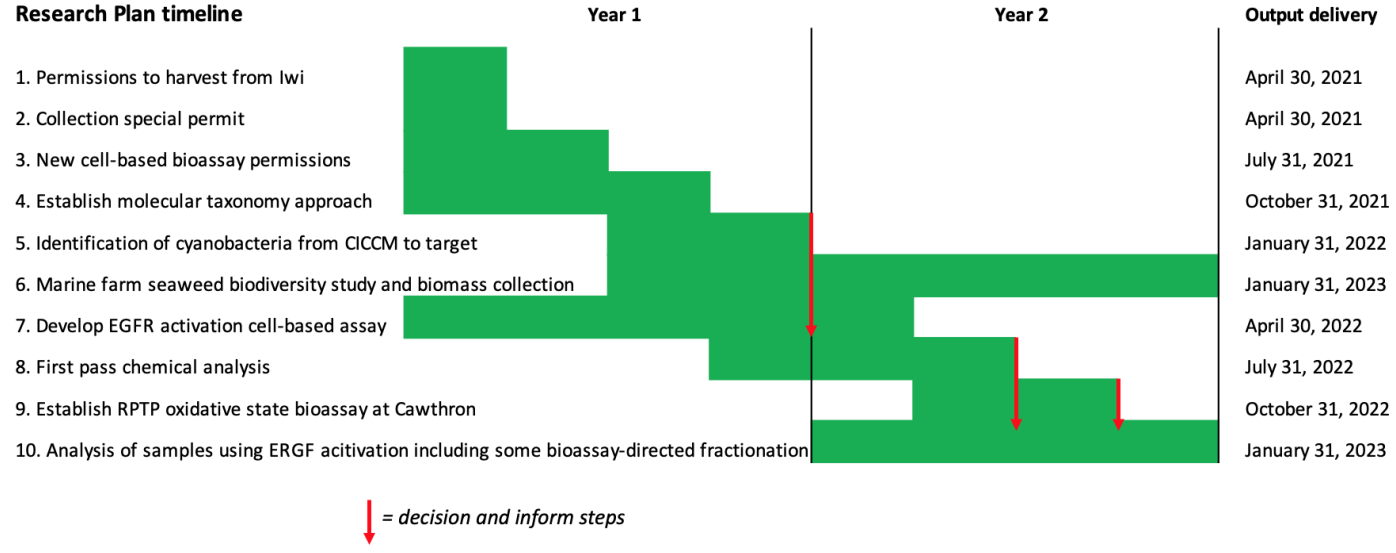
Dr Tom Wheeler, Dr Rita Lee and Dr Jonathan Banks have expertise in protein analysis and western blotting, recombinant technology and expression of recombinant proteins, respectively. Lee and Banks are currently employing molecular taxonomy in their work. A significant proportion of the taxonomic identification of seaweed species will be carried out by Dr Paul South using morphological characteristics, who also has experience designing and executing seaweed ecology field work. South is a seaweed ecologist with the taxonomic knowledge required for this work. Dr Jonathan Puddick, a skilled analytical and natural products chemist, leads the chemical extraction and fractionation and chemical analysis components of the project [53-56].

This project has been co-developed together with our industry partners Wakatū Incorporation and SRW Laboratories Ltd; both integral to the research. The relationship built ensures complementarity of contributions and also means outputs will meet each party's need to develop the discoveries. Wakatū Incorporation will not only supply resources (biomass) through our relationship but intend to proactively take-up results of the programme and help to commercialise them through their new business, Auora. SRW Laboratories Ltd. will feed in knowledge, market insight and resources and inform research decisions.

We are uniquely positioned to carry out this work on NZ algal species because we have tools, team and tactics and this will aligns with the goals of the Blue Economy's core projects to indigenise Aotearoa's blue economy and build a NZ seaweed sector.

The research and commercialisation will be through partnership with Māori and will generate new indigenous enterprises that create value from sustainable wild-harvesting of natural resources either within their rohe or covered under WAI262. The research will identify which species are best for sunscreen protection and Māori can then look at how best to farm these species as part of their commercialisation pathway and providing an avenue for Māori to diversify species in aquaculture.

The research project will begin February 1, 2021 and proceed per the timeline in the Research Plan below and as indicated in the milestone output delivery dates (and in Section O).



I. CONTRIBUTION TO BLUE ECONOMY IN AOTEAROA NEW ZEALAND

This project develops innovative next-generation suncare products from Aotearoa's algal biota resources adding value to our seaweed species able to be grown on marine farms. This aligns with the goal of the Blue Economy to create economic value from marine activities, and contribute positively to social, cultural and ecological wellbeing. The products developed in this project could help to enhance ecosystem health globally as our novel suncare concepts are likely to cause less harm to the environment than current sun blocking agents. The ecotoxicological properties of our discoveries will be confirmed during further commercial development, outside this initial project, once the bioactivity of specific extracts has been established.

Beyond our specific industry partners, the knowledge generated in this project will benefit the regional communities by helping to develop a seaweed aquaculture industry in Aotearoa. It will improve livelihoods by providing employment, and supplement the revenue streams of other aquaculture industries (where multi-trophic aquaculture systems are adopted) reducing investment risk through diversification. Ecologically, seaweed aquaculture captures carbon dioxide and regulates ocean pH in the vicinity, providing benefit to nearby shellfish[57]. As seaweed draw their nutrients from the water they grow in, there are also opportunities for the restoration of marine environments impacted by high nutrient levels (e.g., from industrial activities, agriculture or fish farming) through the inclusion of seaweed aquaculture, whilst generating a revenue stream. With many Māori enterprises already involved in aquaculture and fisheries, opportunities in our fledgling seaweed industry will be readily taken up by organisations that will work with hapū in the regions of operation. This is evident from Wakatū's active support of not only this project but also others related to developing a seaweed industry for the betterment of tangata whenua and the environment.

This programme will provide a model to spur further investment in this area which has great promise for economic, social, cultural and environmental benefits. There is a substantial opportunity for product improvement in the sunburn prevention and treatment category.

Ultimately, delivery of this project will lead to considerable contribution to the goals of the Blue Economy Challenge based on high-value compounds derived from NZ algal bioresources. If successful our next-generation suncare approach **dramatically broadens the scope for human benefit and the commercial opportunity** arising from a new generation of sun-defence products based on NZ algae. The cost of acute sunburn treatment alone is in the billions of dollars per annum excluding indirect costs skin cancer treatment and skin aging resulting from sunburn and UV damage [4-7], with NZ and Australia having the highest rates of skin cancer in the world[8]. Products based on previous technology were valued at NZ$11.7B PA globally in 2010[58], worth NZ$2.8B in the US alone in 2016[59] and are projected to grow a further NZ$4B by 2022[1]. NZ has the opportunity to create a step change in the large market currently based on products aimed only at prevention, delivering more efficacious prevention with fewer side-effects.

J. BENEFITS AND CONNECTIONS TO IWI, HAPŪ AND MĀORI ORGANISATIONS

This programme draws on the traditional role of algae in rongoā Māori (medicine) – algae and particularly seaweeds have played an integral role in Māori communities for generations[60, 61]. This programme has been co-developed with Wakatū Incorporation, as part of its Indigenous Organisms Programme and the development of their new brand Auora[62]. Wakatū Incorporation is owned by 4,000 Māori families who descend from the traditional landowners of Nelson, Motueka and Golden Bay. They have interests in aquaculture and high-value natural products. Wakatū plans to develop two higher-value bioactives with validated efficacy within the next five years. Wakatū's interests include immune health and skincare.

Cawthron has a long-standing and multi-faceted relationship with this organisation covering seaweed as a high-value food as well as aquaculture-based production of native species, and Wakatū is well placed to create impact from the research. Specifically, Wakatū will carry out a biodiversity study on their marine farms and other collaborators which span the length of the South Island of NZ, importantly covering different biophysical areas from Stewart Island to the Marlborough Sounds. They will also provide seaweed biomass for our research. Finally, they will lead the commercialisation phase, following on from the research programme. This includes development of systems for commercial harvesting and processing, regulatory compliance requirements, market evaluation, and business case development. The next-generation suncare product concept fits well with Wakatū's strategy of maximising the value

of its significant aquaculture resources through their knowledge systems (mātauranga Māori) and conventional science to develop high-value products that improve the economic, health, environmental and cultural outcomes. Moreover, the programme leverages Wakatū's experience and understanding of target markets and how to integrate Māori values into premium offerings, which includes other product concepts based on seaweed. Wakatū is heavily invested in seaweed research, including support of a HVN programme in this area, and has expressed strong interest in developing this value chain.

Additional benefit to Māori more generally will come through new employment opportunities within the value chain, including harvesting, processing and marketing, which will all be controlled at the iwi or hapū level. Finally, Māori will benefit from the increase in value of seaweed resources under their jurisdiction. Overall, the programme draws on the Vision Mātauranga theme of Taiao, achieving environmental sustainability, by ensuring the scale of harvesting does not affect marine biodiversity as well as providing an environmentally-friendly alternative to the contaminating petrochemicals used in current sunscreens.

See also document "Wai262 and benefits to Māori Response, Algae Skin Sun Defence" supplied separately.

K. COMMUNICATION OF PROJECT RESULTS

Information generated in this project will have commercial sensitivity associated with it. To ensure that maximum benefit to NZ is realised, communication of results will be overseen by a steering committee comprised of Cawthron Business Development personnel, Cawthron's patent attorney and representatives from Wakatū Incorporation and SWG Laboratories Ltd. The steering committee will meet with the research team at least two times a year, but potentially more frequently when specific matters regarding commercialisation of the science needs to be addressed. Once IP protection is secure, results will be communicated through hui, media and scientific publications.

Outcomes from the project will be communicated to stakeholders through hui. This will allow the interested hapū/iwi that form the shareholder-ship of Wakatū Incorporation to engage with and feed into the research.

We will highlight this science to a wider audience through media stories and radio, TV, print and digital forums. We aspire to not only highlight the science, but also the cultural, social and environmental importance of the work being undertaken. Cawthron's Communications and Māori Business Development teams, alongside our industry partner communications teams will assist the research team on this endeavour.

Scientific results, that will not provide outside commercial entities with the ability to easily duplicate products and formulations, will also be published in peer-reviewed journals. This will provide extra credibility for the products being developed and contribute new scientific knowledge (e.g., the taxonomic diversity of NZ seaweed).

L. CO-FUNDING

Co-funding for this project comes from our industry partners:

Wakatū Incorporation will manage, negotiate access and execute the biodiversity study of seaweed on marine farms following our guide on design and methods for sampling and processing of seaweed material.

SRW Laboratories Ltd, will provide support for screening of the extracts from algae once our methods have been established. Furthermore, they will provide market insight and perform work to investigate formulation of skin products based on candidate algae extracts as they come to hand through the project. This is based on their experience with different formats suitable for outcomes of the research.

M. RISK & MITIGATION

Risk	Mitigation
Biosecurity	Cawthron has procedures for handling biological materials to reduce to mitigate biosecurity risks.
Not able to identify compounds with desired properties from algae	We seek multiple modes of action from algae. The ideal outcome is discovering compound specifically protecting RPTP. We've identified the activity in an overseas brown seaweed[38] indicating that this is probable in NZ species. Similarly, we are confident that MAAs will be found in NZ algae.
Intellectual property	We will adapt successful IP arrangements in place for similarly-structured projects at Cawthron incorporating WAI262 principles. New IP will stay in NZ.
Commercial risk	We will adapt successful IP arrangements in place for similarly-structured projects at Cawthron incorporating WAI262 principles. The IP will stay in NZ.
COVID-19	This project is not dependent on regular face-to-face communication. COVID-19 may disrupt travel, so we will train staff at marine farms on sampling regimes for supply of algal material.

N. CONSENTS & APPROVAL required to undertake research

Cawthron and our agents have the necessary permits from the Ministry of Primary Industry (MPI) for collecting material including algae for research purposes. This project will be added to Cawthron's special permit (SP651-6). Appropriate regional authorities including local iwi and/or regional councils will be engaged prior to conducting collections. MPI will be contacted prior to conducting any collections to ensure that biosecurity risks are managed.

EPA permitting for recombinant expression in mammalian cell lines for the new scalable bioassay will be obtained if necessary, however we will endeavour to develop the assay in a cell line that already expresses RPTP and EGFR which will not require permitting. We have PC2 facilities and have had these certifications previously.

o. References

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