

A. PROJECT TITLE

Innovative technologies for the early detection of harmful Algal Bloom (HAB) threats

B. PROJECT TEAM

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C. ABSTRACT

Harmful algal blooms (HABs) are natural phenomena that significantly impact many ecosystem services and values including bivalve and fin-fish aquaculture as well as recreational and customary harvest of kai moana.

We propose to introduce to New Zealand and experimentally trial two complementary innovative technologies (the **Hydrogel™ qPCR assay** and the **Imaging FlowCytoBot (IFCB)** instrument) for the detection and monitoring of harmful planktonic micro-algae. This project is applied research aimed at improving the effectiveness, and lowering the cost, of current harmful algae monitoring methods by ensuring the community and aquaculture industry has access to the latest technical advances in this field.

Following essential formatting and experimental validation trials to be carried out under this project, the **Hydrogel™ assay** will provide a fast and effective screening method as an alternative to the current microscopy methods for detecting problem algae species. The assay will be sensitive, simple and robust and able to be carried out in a basic laboratory at sea or in an on-shore processing facility by an appropriately trained person. It will enable the analysis of water samples from numerous sites rapidly and simultaneously, making it more cost effective and time efficient than current microscopy methods.

The application of the **IFCB** has an element of 'blue skies' research and represents a significant step towards the ultimate goal of *in situ*, real-time monitoring robotics that can provide near instantaneous warning of potential harmful algal bloom events. The **IFCB** is an autonomous submersible microscope that continually photographs and counts microscopic particles in seawater as it is pumped through the instrument. Using image recognition software the instrument can automatically identify and count individual species in the plankton and

provide an alert when problem species appear and increase in number. To achieve this, a period of machine ‘training’ is required to enable the instrument to encounter and recognise the size and morphology of local planktonic organisms from multiple viewpoints. The current instrument prototype is expensive and is undergoing experimental trials around the world. We have a unique opportunity to trial an **IFCB** in New Zealand (at no instrument cost) to evaluate its potential to accurately identify and estimate the abundance of local harmful micro-algae species. It is expected that future versions of this instrument will be useful in remote offshore aquaculture situations for continuous *in situ* plankton monitoring.

During the **Hydrogel™** assay development and calibration phase we will primarily use micro-algal cultures from the Cawthron collection and seawater samples from the Marlborough Sounds where we know that target species occur. Subsequent field trials will be carried out in other aquaculture regions with chronic HAB problems such as the Bay of Plenty. For the software learning phase of the **IFCB** trials, algal cultures and seawater samples from various locations in the Marlborough sounds containing representative phytoplankton assemblages will be used. Approximately 1 month *in situ* field trials will be carried out at a Marlborough Sounds salmon farm and at least one other location where harmful algal blooms regularly occur.

D. RELEVANCE TO CHALLENGE OBJECTIVE

The research is closely aligned with the Challenge objective (“Enhance utilisation of our marine resources within environmental and biological constraints”) by developing and trialling innovative tools for mitigating important natural hazards that have the potential to constrain the financial viability, sustainability and diversification of the aquaculture industry. In addition it will enable unique insights into plankton community structure, life cycles and ecology within aquaculture regions, which is essential to a scientifically grounded ecosystem-based management approach. Product certification and provenance, is identified in the Challenge as a key pathway by which value will be added to the marine economy. However this vision will only be achieved if biological hazards are mitigated and the safety of seafood products on local and export markets is guaranteed by continual improvement of quality assurance technologies. The project meets the objectives of the Innovation Fund in bringing new innovative approaches capability, research and researchers to the Challenge.

E. INTRODUCTION

The proposed research **aims to develop innovative technologies for detecting harmful phytoplankton species in near real time**, thereby enabling rapid response of Industry and other stakeholders to the onset of HABs, and where possible, mitigate their impacts. New Zealand finfish aquaculture is currently a ~\$130 million industry (2011) with scope for expansion of salmon farming in cooler South Island waters, and the potential for the rearing of other species in warmer regions, to generate new export earnings and employment (1). Bivalve farming is well established in the North and South Islands and is poised for substantial expansion in offshore waters such as the Bay of Plenty, Nelson Bays, Pegasus Bay and Hawkes Bay. Capturing these opportunities requires careful mitigation of the risks of farming in an uncontrolled environment.

Catastrophic examples of HAB effects in New Zealand include the near extinction of the nascent salmon industry (NZ\$17 million loss) by *Heterosigma akashiwo* in 1989 (2,3), extensive mass mortalities of marine fauna (paua, surf clams, fish) due to a bloom of *Karenia* spp. on the coast of Southland in 1994 (4), mass mortalities of marine flora and fauna in Wellington Harbour in 1999 due to a bloom of *Karenia brevisulcata* (5), and the loss of several million dollars’ worth of

salmon on NZKS's Ruakaka, salmon farm in 2010 due to a bloom of *Pseudochattonella verruculosa* (6). There have also undoubtedly been many more of these events in more remote areas which have passed unnoticed and problems of this nature may be expected to increase with projected climate change and expansion of aquaculture into new areas. The recent event (Feb-Mar 2016) that caused the loss of US\$ 500 million worth of cultivated salmon in Southern Chile (7) was due to a bloom of *P. verruculosa* which was clearly associated with an unusually strong El Nino-mediated climate anomaly.

Contamination of bivalves with a variety of micro-algal biotoxins has caused major problems for shellfish aquaculture and public health in New Zealand (8). From the point of view of food safety and export market security, it is vital that toxic bloom events are detected at the earliest possible opportunity. Overseas consumers and regulators are acutely sensitive to food contamination issues and the consequences of algal-toxin contaminated product being inadvertently exported could be disastrous. For example, all Australian shellfish exports (bivalves and crustaceans) were banned in 2012-13 when undetected PSP-toxin contaminated mussels from Tasmania were exported to Japan (9). This resulted in an economic impact of over \$A23 million to the Australian marine farming and fishing sectors. In this context the large offshore farms being established in the Bay of Plenty are especially at risk. This region has a long history of PSP-toxin problems and cases of human poisoning. In Nov-Dec 2016 Whakatohea Mussels' large offshore farm in the Bay of Plenty was closed due to high levels of PSP toxin. The size and remoteness of the offshore farms creates difficulties for rigorous monitoring and particular vigilance is essential for ensuring undetected contamination of harvested product does not occur.

F. AIMS

We aim to apply two complementary innovative technologies to provide near real-time data, on the population dynamics of harmful phytoplankton that impact aquaculture. These rapid, high precision methods will enable critical early warning of shellfish biotoxin contamination and timely implementation of mitigation measures on fin-fish farms (cessation feeding, deployment of airlifts etc.). We address project 2.2.2 ("*Methods to increase diversification in marine economies*") since there is a proven high risk from algal blooms in northern areas (e.g. Hauraki Gulf, Bay of Plenty) where diversification and expansion of the industry is likely. The proposal has clear connections between research aims and commercial activities. It is aimed at the development and application of technologies for improved water column monitoring relevant to aquaculture, and leverages off International capability for the benefit of local industry that will provide in kind support for field trials. The proposal is highly relevant to the interests of Māori, who own major companies (e.g. Sealord, Wakatu (Kono), Ngāi Tahu Seafood, Whakatohea-Eastern Seafarms Ltd.) with numerous marine farms across New Zealand. The technologies we will introduce have potentially wider applications, such as the rapid identification and enumeration of other planktonic organisms of relevance to aquaculture (e.g. bivalve larvae, biofouling species).

G. PROPOSED RESEARCH

We propose to apply two new complementary technologies to provide near real time data, at aquaculture farm sites.

Technology 1. Development of **HydrogelTM** tests for harmful algae The first technology is a field deployable qPCR assay developed by Aquila Diagnostic Systems, Edmonton, Canada (<http://www.aquiladiagnostics.com/>) with its primary applications in the veterinary and human health (e.g. Ebola, Malaria) diagnostic areas. Cawthron researchers have worked collaboratively with scientists at **Aquila** for some time, and the efficacy of this technology for the diagnosis of

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oyster herpes virus infection has been demonstrated in New Zealand (10) and found to offer a viable, low cost method of conducting OsHV-1 assays in the field. The **Hydrogel™** instrument is capable of performing PCR in the field with minimal operator input and training. The unique feature of the **Hydrogel™** instrument is that reagents are stabilised by polymerisation in a gel matrix which means the usual refrigeration of reagents is not required. As the gels are pre-prepared, operator input is minimal and only requires basic facilities. Because the reactions are sealed, the risk of contamination is reduced and can be carried out almost anywhere with rudimentary laboratory facilities. The research will involve the transfer of developed qPCR assays that target the most important toxic marine HAB species in New Zealand, to the **Hydrogel™** format. We will specifically target DNA sequences unique to the ichthyotoxic species *Pseudochattonella verruculosa*, *Heterosigma akashiwo* and *Karenia brevisulcata*, and the paralytic shellfish toxin producers *Alexandrium catenella*, *A. ostenfeldii* and *A. minutum*. This work will be carried out by Cawthron staff at the laboratory of Aquila Diagnostic Systems. Appropriate diagnostic DNA sequence data from New Zealand isolates of these species are already available and live cultures for testing and calibration of the assays are held in the Cawthron Institute Culture Collection of Micro-algae. A simple and rapid plankton sampling and DNA extraction method that can be performed in a basic on site facility will be developed.

The work plan involves:

- Development of primer sets for selected harmful algal species from available DNA sequence data either from our own past research (11) or from the literature (12) and where necessary *de novo* sequencing of cultured isolates.
- Fabrication of reaction gels tailored for the identification of individual target species at **Aquila Diagnostic Systems** (by Jonathan Banks).
- Transfer and optimisation of developed qPCR assays to the **Hydrogel™** format
- Experimental validation/calibration trials involving comparison of cell counts by microscopy and flow cytometry to quantification using the **Hydrogel™** assays on cultures and natural seawater samples. Archived time-series plankton concentrates from past bloom episodes are also available for validation purposes.
- Demonstration of the **Hydrogel™** assay to industry users in a field setting including a NZ King Salmon farm site and Whakatohea Mussels on shore landing facility.

We expect this technology to be rapidly adopted once experimental trials and validation are completed.

Technology 2. Imaging FlowCytobot trials

The **Imaging FlowCytobot (IFCB)** is an-autonomous submersible, *in situ* imaging flow cytometer, manufactured by McLane Labs, USA (<http://mclanelabs.com/imaging-flowcytobot/>). It combines flow cytometry and video technology to capture images of individual cells. Laser-induced

fluorescence and light scattering from individual particles are measured and used to trigger targeted image acquisition; the optical and image data are then transmitted to shore in real-time. The IFCB generates high resolution (1 µm) images of suspended particles in the size range <10 to 100 µm

(such as diatoms and dinoflagellates). Images collected during continuous monitoring are processed with image classification software. After training the software, the images can be automatically classified to provide, identification and abundance and bio-volume estimates.

The instrument continuously samples at a rate of 15ml of sea water per hour and can generate on the order of

30,000 high resolution images per hour. Organisms can be classified to the genus or species level with an accuracy comparable to that of human experts. This enables real time reporting of species abundance, biomass and community composition.

Long term deployment of the **IFCB** has provided unique insights into the dynamics of phytoplankton communities elsewhere (13). On the east coast of the USA multi-year trends in blooms have been linked to regional climate related variables and in the Gulf of Mexico the **IFCB** has provided successful early warning of numerous HABs. Initial stages of these blooms have been detected with sufficient time to close shellfish harvesting and prevent human illness (14). High resolution time series data from the **IFCB** has been used to develop models to determine the origin of HAB populations and forecast their occurrences (15). The **IFCB** technology is undergoing continued development and improvement but already it can be deployed for extended periods unattended. It may provide the solution to the monitoring of phytoplankton in large scale, remote offshore aquaculture areas that cannot be adequately monitored using manual sample collection and microscopic analysis.

We have the opportunity through collaboration with Professor Raphael Kudela, University of California at Santa Cruz to trial a **IFCB** instrument at no instrument cost in the Marlborough Sounds over the high-risk HAB period in summer 2017-18.

The work plan includes:

- Calibration and training of image recognition software in the laboratory using algal cultures from the Cawthron Culture Collection of target species, and raw water samples containing common local phytoplankton
- A one month field deployment of the **IFCB** on a NZ King Salmon Farm in Pelorus or Queen Charlotte Sound to build an image library of local species, monitor HAB species and demonstrate the technology to industry for real time visualisation and analysis.
- A one month field deployment in Opuia Bay Tory Channel during the growth and climax phases of the annual toxin producing *Alexandrium catenella* bloom (8). The aim is to obtain data on timing of life cycle transitions and estimation of *in situ* growth rates for parametrisation of biophysical bloom prediction models.
- Analysis of deployment data and preparation of a publication on application of this technology in New Zealand.

These trials represent a *proof of concept* component of the project, laying the ground work for adoption by industry of future iterations of the technology.

H. RESEARCH ROLES

Researcher	Organisation	Contribution
<i>Lincoln MacKenzie</i>	<i>Cawthron</i>	<i>Project management, field trials, phytoplankton identification/taxonomy</i>
<i>Jonathan Banks</i>	<i>Cawthron</i>	<i>Design and fabrication of Hydrogel™ matrices containing</i>
<i>Kirsty Smith</i>	<i>Cawthron</i>	<i>Design of primers for Hydrogel™ assays, development of</i>
<i>Raphael Kudela</i>	<i>UOC</i>	<i>Setting up and operation of Imaging FlowCytoBot instrument</i>

Ben Knight	Cawthron	Customisation of Imaging FlowCytoBot image analysis machine learning software
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I. LINKAGES AND DEPENDENCIES

The proposed research complements a number of projects already in progress within several of the Challenge's programmes including:

Our Seas: 1.2.1 Frameworks for achieving and maintaining social licence. An important factor in achieving social licence is convincing the public that there is a good understanding of the consequences of commercial operations in the marine environment and monitoring of potential effects. Because of the highly dynamic and variable nature of biological processes in the water column empirical (i.e. as opposed to modelling) approaches to satisfying these needs is very difficult to achieve. The **IFCB** technology that we will introduce is one of the few means available of monitoring farm effects by providing the ability to observe plankton community dynamics autonomously, *in situ* and in real time over extended periods.

Valuable Seas: 2.1.3 Measuring ecosystem services and assessing impacts. The technologies we will introduce enable a much improved ability to assess the biodiversity and ecology of the plankton which globally and locally underpins the majority of marine ecosystem services.

Managed Seas: 5.1.1 Ecosystem models Marine ecosystem models are valuable tools for exploring the broad scale consequences of developmental pressures, management practises and as guidance to support decision-making processes. However the complexity and dynamics of plankton communities and water column processes cannot be modelled with any degree of certainty at the species level. The resolution of the technologies we will introduce start at the scale at which ecosystem models cannot provide meaningful information, for example on the phenology of phytoplankton and zooplankton communities. This information will augment ecosystem model outputs, and can be used in validation of biogeochemical modelling aimed at assessing aquaculture- environmental interactions, such as the effects of finfish farms on primary production and HABs.

Dynamic Seas: 4.1.1 Tracing biochemical fluxes to inform ecosystem based management, 4.2.1

Tipping points in ecosystem structure, function and services, 4.2.2 Stressor footprints and dynamics. These projects seek to understand how ecosystems function and respond to human disturbances by following the flow and fate of organic matter and nutrients in marine food webs; model the dispersion of contaminants to assess the near and far field effects of marine activities and examine how subtle but cumulative impacts can reach "tipping points" that profoundly change the nature of marine ecosystems. None of these studies address the important issue of regime changes in the floristic composition of the phytoplankton and micro-zooplankton that can have profound

flow on effects on ecosystem function and for which our technologies are ideally suited. There is potential to align outputs using the IFCB in the Marlborough Sounds with water column sampling planned for cruises within Project 4.1.1 which aim to study biochemical and planktonic fluxes between aquaculture and the wider marine ecosystem.

Lastly, our proposal has links with a current Innovation Fund project ("**Open for Business: near real- time forecasting to reduce harvest closures**") on predicting bacterial contamination risk in shellfish growing areas. The application of the **Hydrogel™** assay will

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provide proof of concept for developing future tests for rapid enumeration of faecal indicators used to manage harvest closures.

Public health and shellfish industry biotoxin monitoring programmes complement each other by providing spatial coverage beyond their specific areas of responsibility and extensive data sharing arrangements exist between various organisations (e.g. Marlborough District Health Board and Marlborough Shellfish Quality programme). Therefore any improvements in monitoring technologies adopted by the industry will also inevitably improve security for non-commercial interests. The situation in the Bay of Plenty (BOP) provides an excellent example. Paralytic shellfish poisoning is a proven health hazard to consumers of shellfish (mainly surf clams) gathered from BOP beaches, however because of the nature of the coastline (predominantly exposed surf beaches), it is difficult and expensive to obtain seawater samples that are truly representative of offshore phytoplankton communities. For this reason early warning of contamination by phytoplankton monitoring has always been less reliable in this area than in others (e.g. the Marlborough Sounds). The establishment and technology improvements to routine monitoring of offshore marine farms in the BOP will enhance the early detection of algal bloom events that may impact publically accessible inshore surf clam resources.

J. RISK AND MITIGATION

It is not expected that there will be a significant risk of not succeeding in transferring qPCR assays for HAB species to the **Hydrogel™** format, although like any molecular biology procedure unexpected interferences occasionally occur that may need to be overcome by the modification of standard procedures. A streamlined protocol for phytoplankton concentration and DNA extraction will need to be developed, but no technical obstacles to achieving this are expected.

As with all sophisticated instrumentation, particularly moored *in situ* monitoring devices in the harsh marine environment, there is always the risk of instrument failure with **IFCB**. However these instruments have been in operation for several years and have proved very reliable with proper maintenance. Prof Kudela has extensive experience in their operation and deployment, and in the event of a technical failure that is beyond our capabilities to fix, the manufacturer (McLane Labs) can be called upon. Cawthron also has specialist expertise and a growing track record in the development and deployment of real-time instrument platforms.

Decades of nation-wide routine phytoplankton monitoring in New Zealand has proven time and again that analysis of water samples collected from well-chosen locations does provide a reliable indicator of spatial and temporal changes in community composition and an early warning of impending harmful algal blooms. The technologies we plan to introduce directly address the issue of phytoplankton spatial heterogeneity, which we emphasize in our proposal may be a significant issue with the large scale offshore marine farm developments currently underway. A major strength of the **Hydrogel™** assay is that it will provide an effective screening method that will enable the analysis of water samples from numerous sites rapidly and simultaneously giving greater spatial coverage faster and more cost effectively than current microscopy methods. The Imaging **FlowCytobot (IFCB)** trials are more spatially constrained by only having a single instrument though this is less of an issue with sea cage monitoring since these are much less dispersed than shellfish farms. Vertical profiling and horizontally mobile deployment of the **IFCB** (e.g. from a vessel underway) are possible and future iterations of this technology may employ

autonomous drones programmed to independently sample numerous locations over large areas.

K. ALIGNED FUNDING AND CO-FUNDING

Cawthron is a major research provider and participant in national public health and aquaculture- phytoplankton and biotoxin monitoring programmes (16). The project will complement research under Cawthron's existing 'Safe NZ Seafood' programme (MBIE contract CAWX1317). The 'Harmful Algae Ecology' objective in the programme (\$137K/annum) is focused on identifying the environmental and biological drivers of recurrent toxic dinoflagellate blooms in the Marlborough Sounds. An important element of our proposal on improved methods of early warning of fish killing species, is outside the scope of the current MBIE programme. The project will be integrated with the current monitoring programmes carried out by Cawthron under contract to the Marlborough Shellfish Quality Programme (MSQP), NZ King Salmon Co. Ltd., MPI and Marlborough District Council. NZ King Salmon (Resource and Environmental Manager Mark Gillard; General Manager Mark Preece) have agreed to assist with the deployment (e.g. provision of platform and power supply) of the **IFCB** instrument from one of their farms. MSQP (Executive Officer Colin Johnson) has agreed to provide logistical support through the provision of phytoplankton samples for testing of assays and instrumentation. This project aligns with Cawthron's strategy to introduce low cost, DNA based monitoring of faecal indicator bacteria as part of the 'Safe NZ Seafood' programme and the NSC project: "Near real-time forecasting of contamination risk to reduce commercial shellfish harvest and beach closures" (\$400k).

Our proposal is closely aligned with Cawthron's "Enabling Open Ocean Shellfish Aquaculture" (MBIE contact CAWX1607; \$5.3 million) research programme. This programme is aimed at overcoming impediments to the use of over 10,000 ha of unutilised open ocean marine farm space, with potential to double NZ's shellfish export revenue. The programme will develop novel, low maintenance, submersible, flotation systems to enable sustainable and cost effective shellfish farming in the open ocean environment. As these offshore areas are developed there will be a need for improved methods of quality assurance monitoring to meet the challenge of carrying out rigorous and timely harmful phytoplankton surveillance in the open ocean environment. The offshore aquaculture project includes the development and long term deployment of instrument platforms and future versions of the IFCB technology may be added to these installations.

The Cawthron Culture Collection of Micro-algae (recognised by MBIE as a Nationally Significant Database (MBIE contract CAWX0902; \$195K/annum) aligns with the project. It contains a wide range of micro-algae taxa sourced from New Zealand's coastal waters and is essential for the development and validation of species and group specific molecular probes and assays and for the training of imaging software within the **IFCB**.

We have secured a 3-year Royal Society Catalyst Leaders Fellowship grant (\$150,000) to assist with travel and accommodation expenses associated with three annual visits by Prof. Kudela who will bring one of his **IFCB** instruments to Cawthron over the 2017-18 summer. The collaboration with Prof. Kudela (16) in this project will promote International exchange in technical innovation within the Challenge and aligns with MBIE's recent strategic action plan for strengthening Internationalisation of NZ science impact.

For over 25 years Cawthron has been a provider and participant in national public health and aquaculture- phytoplankton and biotoxin monitoring programmes. Cawthron has been

the sole provider of phytoplankton analytical services over this period and for the last 5 years the sole national provider of marine biotoxin analytical services. The development of these capabilities has arisen from Cawthron's public good research which has made it a world leader in this field. Cawthron has a long track record of successfully transferring technologies from research methods to fully validated analytical services used in regulatory monitoring programmes. If these technologies prove successful and of practical benefit to stakeholders, after consultation with the regulator (MPI), they will be incorporated into the current monitoring programmes. Provision for industry demonstrations and training has been included in the proposal budget should end users wish to use the **Hydrogel™** assay independently.

L. VISION MĀTAURANGA (VM)

Māori have mana moana over significant tracts of Aotearoa's coastal and marine environment. As kaitiaki (guardians) of these resources Maori are active players in the protection and management of these ecosystems. A number of Iwi are increasingly important players in the New Zealand aquaculture industry and control a large portion of the 10,000 ha currently consented open ocean water-space. Our research is directly relevant to the viability and sustainability of the Māori aquaculture sector. Under the Vision Mātauranga policy framework this proposal is directly relevant to *Hauora/Oranga*, through improved technologies to detect algal blooms and an expected uptake of this technology to better protect human health in areas of cultural harvest in bloom prone regions (e.g., Hauraki Gulf and Bay of Plenty). We anticipate that this improved technology will also mitigate the financial impacts of bloom events (fall in production and product quality) to Māori aquaculture ventures via real time detection. An industry based on a sound quality assurance and hazard mitigation footing will deliver the employment and financial benefits that will lead to improved health, social and economic wellbeing. *Taiao* benefits will be delivered through a more accurate assessment of bloom events which are also a surrogate indicator of underlying environmental drivers and therefore can inform kaitiakitanga practice and knowledge. Throughout this project we will identify opportunities to engage with Māori (industry players, iwi groups and interest groups) to seek their unique perspectives and explore opportunities within this programme and externally to support their cultural and economic imperatives.

In year 2 of the project we will host a selected Whakatohea Mussels staff member at Cawthron to train in the use these technologies and receive an introduction on how the harmful phytoplankton and marine biotoxin surveillance programmes are carried out and a historical perspective on toxic algal blooms in the Bay of Plenty.

M. CONSENTS AND APPROVAL

No ethics approval, nor any special marine consent is required for this research. Cawthron has two permanent consented moorings in Port Underwood and Opua Bay, Tory Channel that may be used for the *in situ* deployment of the **IFCB** instrument. Approval from NZ King Salmon Ltd has been obtained for the deployment of the instrument from one or more of their farms in Queen Charlotte and Pelorus Sounds.

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