

The 4th International Oyster Symposium (IOS4)

"Embracing the Future through Innovation"

15th ~ 18th September, 2011

Hobart, Tasmania



PRINCIPAL SPONSOR



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ORGANISING COMMITTEE

Dr Katsuyoshi Mori
 Dr Tom Lewis
 Mr Raymond Murphy
 Dr Wayne O'Connor

INTERNATIONAL ADVISORY COMMITTEE

Chairman

Dr Katsuyoshi Mori President, The World Oyster Society
 Professor Emeritus, Tohoku University, Japan

Members - (in alphabetical order of family names)

Dr Nejla Aloui-Bejaoui	Professor, National Institute of Agronomy, Tunisia
Dr Pierre Boudry	Geneticist, Physiologie et Ecophysiologie des Mollusques Marins, IFREMER, France
Dr Kwang-Sik Choi (Albert)	Professor, School of Applied Marine Science, Cheju National University, Republic of Korea
Dr Fu-Lin E. Chu	Professor Emeritus, Virginia Institute of Marine Science, USA
Dr Ximing Guo	Professor, Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, USA
Dr Jonathan W. King	Professor, Centre for Applied Marine Sciences, Bangor University, UK
Dr Balasaheb G. Kulkarni	Director, The Institute of Science, India
Dr René E. Lavoie	Scientist Emeritus, Bedford Institute of Oceanography, Canada
Dr Kuo-Tien Lee	Professor and President, National Taiwan Ocean University, Taiwan
Dr Tom Lewis	Executive Officer, Oysters Tasmania, Australia
Dr Qi Li	Professor, Fisheries College, Ocean University of China, China
Dr Wayne O'Connor	Principal Research Scientist, New South Wales Department of Primary Industries' Port Stephens Fisheries Institute, Australia
Dr David Raftos	Associate Professor, Department of Biological Sciences, Macquarie University, Australia
Dr René Robert	Head, The Molluscs Experimental hatchery of Argenton (Britanny), IFREMER, France
Dr Wei-Cheng Su	Director General, Fisheries Research Institute, Council of Agriculture, Taiwan
Dr Ketut Sugama	Director General of Aquaculture, Ministry of Marine Affairs and Fisheries, Republic of Indonesia, Indonesia
Dr Keisuke Takahashi	Associate Professor, Graduate School of Agricultural Science, Tohoku University, Japan
Dr Aswani K. Volety	Professor, Marine and Environmental Science, Florida Gulf Coast University, USA
Dr Mitsugu Watanabe	President, Watanabe Oyster Laboratory CO., LTD., Councillor, Oyster Research Institute, Japan
Dr Noel P. Wilkins	Professor, Department of Zoology, National University of Ireland, Galway, Ireland
Dr Xinzhong Wu	Professor, College of Animal Sciences, Zhejiang University, China
Dr Mamoru Yoshimizu	Professor, Graduate School of Fisheries Sciences, Hokkaido University, Japan

THEME OF THE SYMPOSIUM

Embracing the Future through Innovation

OBJECTIVES

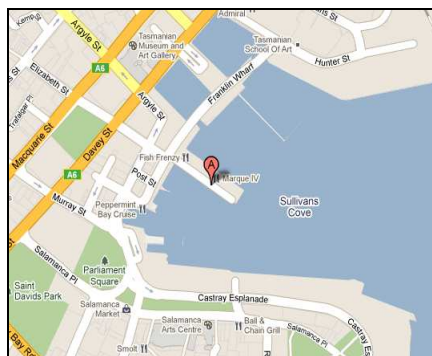
- Innovation in supply
 - Improving hatchery seed supply and seed quality
 - Improving oyster production through breeding programs
- Innovation through diversification
 - Producing better oysters
 - Producing new species
- Innovation in a changing environment
 - Managing risks caused by:
 - Climate change
 - Heavy metals
 - Endocrine disrupting chemicals
 - Biotoxins
 - Oyster diseases
- Innovation in promotion, handling and marketing
 - Improving human health
 - Increasing shelf life
 - Improving retail packaging
 - Building social licence

SCHEDULE

15 September 2011	Symposium Sessions <ul style="list-style-type: none"> • BBQ dinner & farm tour
16 September 2011	Symposium Sessions
17 September 2011	Symposium Sessions - shellfish futures 2011 <ul style="list-style-type: none"> • Symposium and conference dinner
18 September 2011	Post-symposium Tours, or, Free Hatchery Tour

THE SYMPOSIUM VENUE

Hobart Function and Conference Centre, 1 Elizabeth St Pier, Hobart, Tasmania, Australia



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- Austasia Aquaculture Magazine

The organisers gratefully acknowledge the contribution of the Seafood CRC, the Department of Agriculture Fisheries and Forestry, the Australian Centre for International Agricultural Research and Shellfish Culture for their support for delegates to attend IOS4.

KEYNOTE SPEAKERS

Dr Stan Allen

Professor of Marine Science, College of William and Mary, VIMS
Director of the Aquaculture Genetics and Breeding Technology Center, VIMS

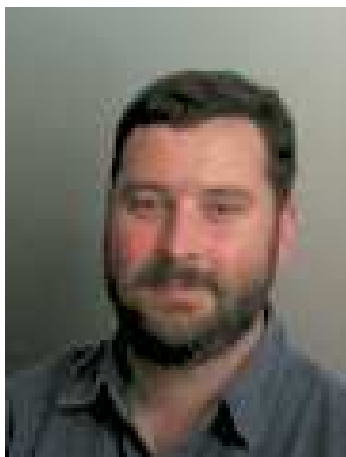


Stan received his BA from Franklin and Marshall College in Biology in 1972, and then went on for a Master's from the University of Maine. He got his Ph.D. at the College of Fisheries at the University of Washington in 1987. His research in Washington was on the commercialization of triploidy, a genetic technique used in domestication of plants and animals, for oyster culture. After a short post-doc at the University of Maryland's Center of Marine Biotechnology from 1987 – 1988, he got a position as Assistant Professor at Rutgers University in New Jersey. At Rutgers he built a breeding and research program at the New Jersey Agricultural Experiment Station. In 1993, he co-developed the process for producing tetraploid shellfish, patented in over half a dozen countries. Stan spent 9 years at Rutgers before coming to the Virginia Institute of Marine

Science in 1998. At VIMS, Stan established and is Director of the Aquaculture Genetics and Breeding Technology Center (ABC), a new research arm at VIMS, its formation enabled by the passage an initiative in 1997 by the General Assembly. Work at ABC has lead directly to the growth of oyster aquaculture in Virginia, which now exceeds wild catch and continues to grow. In an interesting twist of fate, triploids are more popular in Virginia for oyster culture than anywhere else, comprising 80% of production.

Dr Angus Cameron

Director, AusVet Animal Health Services



Angus graduated from veterinary science from the University of Sydney in 1988, and gained his Masters of Veterinary Studies from the University of Melbourne in 1992. He was granted membership of the Australian College of Veterinary Scientists by examination in the area of dairy cattle medicine in 1994. He went on to undertake research for his PhD between 1994 and 1998, developing appropriate surveillance systems for use in developing countries, during which he was based in Thailand and Laos. He joined AusVet Animal Health Services as a Director in 2000. Angus is an Australian epidemiologist with special interest in the areas of surveillance, freedom from disease, health information systems, epidemiological data analysis, geographical information systems, epidemiological training, application of epidemiological

techniques in developing countries and data analysis. He works across of a range of species, including human health, livestock and aquatic animals, in Australia and internationally. In addition to numerous consultancies for government, regional and international organisations, he has been a member of two World Organisation for Animal Health (OIE) working groups responsible for the development of standards for terrestrial and aquatic animal disease surveillance. Angus has extensive training experience ranging from advanced workshops on the analysis of surveillance data through to basic survey skills for field officers in developing countries. He speaks French, Thai, Lao and German at varying levels of proficiency.

Phil Lamb

Managing Director of Spring Bay Seafoods



Spring Bay Seafoods are a Tasmanian based shellfish company operating on the East Coast of Tasmania, 100 kilometres north of the island state capital, Hobart. Spring Bay Seafoods is one of Australia's largest mussel producers, marketers and exporters. Spring Bay Mussels are Certified Sustainable (Friend of The Sea) and Certified Organic (NASAA). The company has won numerous awards for its product business and environmental credentials. Spring Bay Seafoods is the only mussel producer in Australasia to have developed the know-how and capacity to produce juvenile mussel spat in its own bivalve hatchery, located at their land base on the shores of Spring Bay. Spring Bay

Seafoods occupies a unique position as a vertically integrated shellfish company with its own bivalve hatchery producing mussels and recently oysters.

Dr Qi Li

Professor, Associate Dean, College of Fisheries, Ocean University of China, Qingdao, China



Dr. Li received his BSc (1988) and Master (1991) degrees in aquaculture at Ocean University of China. He then obtained his PhD in fisheries in 1997 from Tohoku University, Japan. His postdoctoral work in shellfish genetics was performed at Education and Research Center of Marine Bio-resources, Tohoku University. He became a professor at Ocean University of China in 2000 and established his own research group within Key Laboratory of Mariculture, Ministry of Education. His main research interests include shellfish genetics and breeding, and reproductive biology. He has authored over 120 peer-reviewed papers in these areas.

Lester Marshall

Coffin Bay Oysters



Lester has been oyster farming in Coffin Bay for the past 20 years and has been involved in all aspects of the industry, having built the business up from nothing to some 350 tons of oysters a year and distributing them throughout Australia. He is a Director on the Eyre regional development board and has spent the past seven years developing a regional brand for the Eyre Peninsula. In 2007, Lester applied for and won a Nuffield Scholarship, his study topic being "how to develop a regional brand". He travelled to 15 different countries over the course of this scholarship and completed a report in November 2009. Lester is witnessing the rise of global culinary tourism and is in the process of developing a seafood story book that describes the aroma, texture and flavours of seafood from Eyre Peninsula 'Australia's seafood frontier'.

Dr Len Stephens

Dip Agr Sci, BVSc, MSc, PhD



Managing Director – The Seafood CRC Ltd (Appointed July 2007) A cooperative research centre involving 28 industry and scientific partners investing \$145million over seven years.

Company Director – Dairy Australia Ltd (Appointed November 2007 – November 2010) The principal company responsible for funding and managing research, marketing and trade development for the Australian dairy industry. Fellow of the Australian Institute of Company Directors

Chief Executive Officer, Australian Wool Innovation Ltd (2003 – 2006) With staff in six countries, AWI provides marketing support for Australia's \$3billion Merino wool export industry and conducts research to improve the production and processing of wool.

General Manager, Meat & Livestock Australia Ltd (1996 – 2003). Part of the Executive team that established MLA, the company responsible for research, marketing and promotion on behalf of Australia's beef and lamb producers. Responsible for all the applied livestock production R&D across Australia.

Director, Victorian Institute of Animal Science (1987 – 1996) Inaugural Director of VIAS, established by the Victorian Government to provide veterinary diagnostic services, biotechnology and a wide range of R&D programs.

Bruce Zippel

President, South Australian Oyster Growers Association

President, Shellfish Industry Council of Australia

Past-President, National Aquaculture Council

Founding-President, South Australian Aquaculture Council



Bruce is an active grower in the Zippel family oyster farms at Smoky Bay and Saint Peters Island in South Australia. Has been active in representing the Australia and South Australian seafood industries as well as the oyster industry at a range of levels. A past member of the Premiers Food Council and Aquaculture Advisory Committees in South Australia, as well as being the facilitator and founding director for establishment of the South Australian Oyster Research Council. Bruce is a past member of Australia's Aquaculture Action Agenda Committee, and also chaired the Aquaculture sub-committee of Seafood Training Australia that established the Qualifications Framework and Aquaculture component of the Australia's first Seafood Industry Training package.

SYMPOSIUM PROGRAM *

Thursday 15 September 2011

		Speaker	Title
Chair: Tom Lewis			
8:00	9:00		Registration
9:00	9:05	Tom Lewis	General Welcome
9:05	9:15	Prof Katsuyoshi Mori	Welcome to the 4 th International Oyster Symposium
9:15	9:20	Hon Bryan Green	Opening
9:20	10:00	Keynote 1 Bruce Zippel	The Australian and New Zealand oyster industry
10:00	10:30		<i>Break and poster viewing</i>
Chair: Peter Kube			Topic: Innovation in Supply
10:30	11:00	Keynote 2 Stan Allen	The evolution of and prognosis for commercialization of tetraploid oysters around the world
11:00	11:20	Penny Miller	Development of tools for the sustainable management of genetics in polyploid Pacific oysters (<i>Crassostrea gigas</i>)
11:20	11:40	Sheng-Tai Hsiao	Sequence polymorphism from mitochondrial noncoding region of the Portuguese oyster <i>Crassostrea angulata</i> in Taiwan
11:40	12:00	Tim Green	Genetic immunity and disease resistance in Sydney rock oysters
12:00	13:30		<i>Lunch and poster viewing</i>
Chair: Wayne O'Connor			Topic: Innovation in Diversification
13:30	14:00	Keynote 3 Qi Li	Genetics and breeding of the Pacific oyster in China: progress and prospects
14:00	14:20	Aileen Tan Shau-Hwai	Oyster farming in Malaysia in relation to other Asean countries: challenges and successes
14:20	14:40	Mitsugu Watanabe	Identification of a new anti-oxidant substance from Pacific oyster (<i>Crassostrea gigas</i>) and analysis of anti-oxidant capacity
14:40	15:00	Stephen O'Connor	Advances in hatchery production of flat oyster <i>Ostrea angasi</i>
15:15	22:00		Tas Prime Oysters BBQ dinner and farm tour

Friday 16 September 2011

		Speaker	Title
Chair: Tom Lewis			
8:00	8:30		Registration
8:30	9:00	Doron Ben-Meir	CEO of Commercialisation Australia
Chair: David Raftos		Topic: Risk in a Changing Environment	
9:00	9:30	Keynote 4 Angus Cameron	OsHv1
9:30	9:50	Paul Hick	OsHv1 in NSW
9:50	10:10	David Raftos	Phenoxidase phenotypes are associated with mortality in families of Sydney rock oysters produced by single-pair mating
10:10	10:30	Emma Thompson	Effects of metal contamination on Sydney rock oysters: a proteomic approach
10:30	11:00		<i>Break and poster viewing</i>
Chair: Stan Allen		Topic: Risk in a Changing Environment	
11:00	11:30	Graeme Knowles	The effect of environmental stress on the Pacific oyster <i>Crassostrea gigas</i> : freshwater flooding and the effects of abrupt low salinity
11:30	11:50	Yuki Okada	Multiple mantle lysozymes in the Pacific oyster serve important role for host-defense under broader environmental conditions
11:50	12:10	Naoki Itoh	An abnormal enlargement of the ovary in the Pacific oyster - and old and recurring problem in oyster culture industries of Japan
12:10	12:30	Eric Guevelou	Molecular pathways involved in the gametogenesis of Pacific oysters <i>Crassostrea gigas</i>
12:30	12:45	Vengatesen Thiyagarajan	Oyster larvae are in deep trouble at high-CO ₂ in South China: results of a long-term and large-scale experiment
12:45	14:00		<i>Lunch and poster viewing</i>
Chair: Mark Tamplin		Topic: Innovation in Marketing and Handling	
14:00	14:30	Keynote 5 Lester Marshall	Promotion, handling and marketing
14:30	14:50	Maeva Cochet	Sensory and physicochemical assessment of <i>Crassostrea gigas</i>
14:50	15:10	Anthony Woollams	Selling regional characteristics of wine to the consumer
15:10	15:30	Cath McLeod	Spatial and temporal distribution of norovirus and <i>E. coli</i> in oysters after a sewage overflow into a river
15:30	16:00		<i>Break and poster viewing</i>

		Speaker	Title
		Chair: Len Stephens	
		Topic: General Innovation	
16:00	16:20	Ana Rubio	Monitoring our oysters using automated oyster graders
16:20	16:40	Malcolm Brown	Rapid prediction of oyster biochemical composition using visible-near infrared reflectance spectroscopy (VNIRS)
16:40	17:00	Pham Ahn Tuan/Le Xan	Vietnam oyster industry
17:00	17:15	Tom Lewis	Close

Saturday 17 September 2011**Shellfish futures – Innovations in Industry**

		Speaker	Title
		Chair: Tom Lewis	
8:00	9:00		Registration
9:00	9:15	Tom Lewis	Welcome to 'shellfish futures 2011'
9:15	9:45	Keynote 6 Len Stephens	Innovations from the Australian Seafood CRC
9:45	10:15	Keynote 7 Phil Lamb	Marketing innovations in shellfish - mussels
10:15	10:50		<i>Break and poster viewing</i>
		Chair: James Calvert	
10:50	11:15	Peter Kube	Pacific oyster selective breeding - past, present and future
11:15	11:40	Tom Madigan	Modified atmosphere packaging of half shell Pacific oysters
11:40	12:05	Mark Tamplin	Oyster Shelf-Life = Time and Temperature
12:05	12:30	Shane Comiskey	Benchmarking the Australian oyster industry
12:30	14:00		<i>Lunch and poster viewing</i>
		Chair: Wayne O'Connor	
14:00	15:30	Angus Cameron / Wayne O'Connor (Facilitators)	Workshop (sponsored by Cameron's of Tasmania) "Pacific Oyster Mortality Syndrome (POMS) - industry update and actions"
15:30	16:00		<i>Break and poster viewing</i>
		Chair: Giles Fisher	
16:00	16:15	TSEC Chair	What's happening now in Tasmania
16:15	16:30	NSW OC Chair	What's happening now in New South Wales
16:30	16:45	SAOGA Chair	What's happening now in South Australia

		Speaker	Title
16:45	17:15	Industry Young Leaders group	Busting boundaries: opportunities & challenges
17:15	17:30	Tom Lewis	Close
19:00	23:00		<i>Symposium Dinner & Grand Auction – Hobart Function and Convention Centre</i>

Sunday 18 September 2011

<i>Please note: Each tour time will differ</i>	<i>Shellfish Culture Limited Free Hatchery Tour, or, Post-Symposium Tours</i>
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* This program was correct at the time of printing and is subject to change without notice

SYMPOSIUM POSTERS

15 - 17 September 2011

Presenting Author	Title
Penelope Ajani	Risk-taxa and risk-zones: latitudinal diversity, seasonal periodicity and estuary susceptibility in relation to toxic phytoplankton in the oyster-growing estuaries of New South Wales, Australia
Said Al-Bawani	Heavy metals in rock oysters <i>Saccostrea cucullata</i> and brown mussels <i>Perna perna</i> from the Arabian Sea and Sea of Oman
Matthew Brown	Automation & grading equipment, why and what's next?
Megan Andrew	The Sydney rock oyster <i>Saccostrea glomerata</i> as a biomonitor of estrogenic compounds
Luc Comeau	The effect of silt deposits on the spring awakening of eastern oysters in the gulf of Saint Lawrence, Canada
Michael Dove	Progress, transitions and challenges in the Sydney rock oyster breeding program
Tomomi Hagiwara	Effect of the food containing oyster extract on stress, fatigue and quality of sleep in working persons
Mohamed Houmed Aboubaker	Establishment of stable GFP-tagged <i>Vibrio aestuarianus</i> strains for the analysis of bacterial infection-dynamics in the Pacific oyster, <i>Crassostrea gigas</i>
Yusuke Iidzuka	Genetic diversity of Pacific oyster in Japan
Jens Knauer	Edible oysters culture in tropical Australia
Rhiannon Kuchel	Noradrenaline induces apoptosis of Akoya pearl oyster haemocytes
Dung Le Viet	SWOT analysis - Innovative approach to sustainable oyster culture in Vietnam
Kadek Mahardika	Larvae culture experiment on mangrove oyster <i>Saccostrea cucullata</i> at Bali, Indonesia
Emiko Miki	Clinical efficacy of Pacific oyster extract on sperm profiles in healthy male subjects
Andy Myers	An Industry Led Approach to Managing Risk: Developing EMS in the NSW Oyster Industry
Wayne O'Connor	Cold shock control of biofouling
Wayne O'Connor	Comparative accumulation and depuration of paralytic shellfish toxins in Pacific oysters <i>Crassostrea gigas</i> and Sydney rock oysters <i>Saccostrea glomerata</i>
Bay Phung	Experimental triploid oyster production by chemical induction
René Robert	Improvement of hatchery mollusk seed production: REPROSEED European project
Ana Rubio	Oyster information portal – a novel tool for improved oyster industry management, governance and knowledge
Keisuke Takahashi	Digestive enzyme activities in the digestive diverticula of the pacific oyster, <i>Crassostrea gigas</i>
Daisy Taylor	Effects of metal contamination on the expression of immune- and stress- response genes in Sydney rock oysters
Tomomi Tanaka	Genetic diversity of Suminoe oyster in Ariake Sea, Japan
Chiew Peng Teh	Influence of serotonin and norepinephrine on induction of larvae settlement of tropical oyster larvae, <i>Crassostrea iredalei</i>
Apri Supii	Study of reproductive biology <i>Saccostrea cucullata</i> , in Serangan Coastal, Bali Indonesia

Presenting Author	Title
Lexie Walker	Mudworms in Australia – What’s in a name?
Robin Warner	Oysters in a changing climate: strengthening resilience through effective governance responses
Emma Wilkie	Space Invaders: <i>Crassostrea gigas</i> not presently replacing native oysters along QX disease - infected rocky shores
John Wright	Predicting the physiological response of oysters to climate change
Ziniu Yu	Population genetics of <i>Crassostrea hongkongensis</i> along the coast of South China Sea inferred from mitochondrial genes and microsatellite loci

Please Note: Program is subject to change

SECRETARIAT

The 4th International Oyster Symposium

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website:

www.worldoyster.org/index_e.html

www.oysterstasmania.org

address: c/o RDS Partners
4/29 Elizabeth Street
Hobart. TASMANIA.
Australia. 7000

ASSOCIATED WORKSHOPS

Wednesday 14 September 2011

Industry workshop – Oyster Information Portal; a tool to assist the oyster industry in future planning and adaptation to a changing climate

13:00 – 16:30 Contact: Ana Rubio arubio@uow.edu.au

Sunday 18 September 2011

Australian Seafood CRC Oyster Consortium

Pacific Oyster Mortality Syndrome (POMS) R&D meeting

09:00 – 12:30

ABSTRACTS

Establishment of stable GFP-tagged *Vibrio aestuarianus* strains for the analysis of bacterial infection-dynamics in the Pacific oyster, *Crassostrea gigas*.

Mohamed Houmed Aboubaker*¹, Justine Sabrié¹, Jean-Louis Nicolas¹, and Marcel Koken².

*¹Laboratoire de Physiologie des Invertébrés, UMR 100 PE2M, PFOM, IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France.

²CNRS UMR 6539, IUEM, Université de Bretagne Occidentale, Plouzané, France.

Several marine pathogens are thought to be involved in the summer mortality phenomenon that strikes the stocks of the Pacific oyster (*Crassostrea gigas*) in Europe for more than a decade. Since 2008, mainly a variant of herpes virus, named microvar, is thought to cause the extensive mortalities in juveniles. Therefore the role of the vibrios which are also often detected in the moribund oysters, is less clear. Before 2008 *Vibrio aestuarianus* was detected with a 56% prevalence in these oysters (Garnier et al. 2008) and subsequent laboratory challenges proved its involvement in oyster death. The mechanisms however by which this pathogen enters the oyster and transmits in-between specimens is thus far almost unknown.

To establish genuine model strains which allow the detection of the bacteria during the first hours of an infection, both a highly pathogenic strain (02/41), and a weakly pathogenic strain (01/308) were transformed with green fluorescent protein-expression vectors containing also a Kanamycin-resistance gene.

The clones obtained were compared to the parental strains for their growth characteristics, basic metabolism, antibiotic-resistances and virulence (cumulative mortality). The 02/41 derivative was in all aspects indistinguishable from the parental strain. In contrast, in the 01/308 strain, GFP expression led to a significant increase of virulence. By flow-cytometry, these GFP strains can be easily quantified in seawater and oyster haemolymph, and their in situ detection will allow detection of the bacterial tropism inside the oyster tissues.

Risk-taxa and risk-zones: latitudinal diversity, seasonal periodicity and estuary susceptibility in relation to toxic phytoplankton in the oyster-growing estuaries of New South Wales, Australia

Penelope Ajani*¹, Steve Brett², Martin Krogh³, Grant Webster⁴ and Leanne Armand¹

*¹Climate Futures at Macquarie, Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia *Corresponding Email: Penelope.Ajani@mq.edu.au
Ph: +61-2- 9398-4725 Fax: +61-2-9850-8245

²Microalgal Services, 308 Tucker Road, Ormond VIC 3204, Australia

³Waters and Coastal Science Section, New South Wales Office of Environment and Heritage PO Box A290, Sydney South NSW 1232, Australia

⁴NSW Food Authority, 1 Macquarie Street Taree NSW 2430, Australia

The spatial and temporal distribution of harmful phytoplankton was examined in the oyster-growing estuaries of New South Wales. Forty-five taxa from 30 estuaries were identified from 2005 to 2009. Diversity was latitudinally graded, with taxa increasing southward. Of the 22 estuaries tested for site differences, multivariate analyses revealed significant differences in species abundance and occurrence for 11 estuaries. Differences were predominately due to the causative species, *Pseudo-nitzschia delicatissima* group, *Dinophysis acuminata*, *Dictyocha octonaria* and *Prorocentrum cordatum* with a consistent upstream versus downstream pattern emerging. Phytoplankton seasonal distribution was variable across estuaries but suggested a winter minima. Multidimensional scaling

(MDS) revealed toxic phytoplankton abundance patterns correlated with *mean annual rainfall* and *estuary modification level*, and species occurrence with *estuary class*.

Phytoplankton Action Limits (PALs) were exceeded at 86% sites. High-risk estuaries were identified as Wagonga Inlet, Wallis Lake and Hawkesbury River. Twenty-three taxa exceeded the PALs across all estuaries, the majority of species belonging to *Pseudo-nitzschia*, *Alexandrium* and *Dinophysis*. PALs were also exceeded across all seasons with a winter minimum. How these risk-taxa and risk-zones change with progressive climate warming, coupled with the need for further taxonomic, toxicological and molecular investigations into key taxa (eg. *Pseudo-nitzschia*), are important considerations for future aquaculture in east Australian waters.

Heavy metals in rock oysters *Saccostrea cucullata* and brown mussels *Perna perna* from the Arabian Sea and Sea of Oman

S.M. Al-Barwani*¹, J.S. Goddard¹, B.Y. Kamaruzzaman², K.C.A. Jalal², S. Shahbudin², and M.S. Mohd Zahir²

*¹Department of Marine Science and Fisheries, Sultan Qaboos University, P.O. Box 34, Al-Khodh 123, Sultanate of Oman, Email: sharhi@squ.edu.om

²Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmed Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

Heavy metal concentrations were studied in two different bivalve species, the rock oyster *Saccostrea cucullata* and the brown mussel *Perna perna*. The rock oyster was collected at three sites along the whole coastal areas of the Sultanate of Oman; while the indigenous brown mussel was collected at three sites along the Arabian sea. More than 100 individuals of both bivalve species were sampled and analyzed for metals such as Cadmium (Cd), Copper (Cu), Iron (Fe), Lead (Pb) and Zinc (Zn) using Inductive Coupled Plasma Mass Spectrometry (ICP-MS). The capacity of accumulating Cu, Fe and Zn was greater in *S. cucullata* than in *P. perna*. Lead (Pb) accumulation in both bivalve species were low and at acceptable levels for human consumption.

The evolution of and prognosis for commercialization of tetraploid oysters around the world

Standish K. Allen, Jr.*

*Aquaculture Genetics and Breeding Technology Center, Virginia Institute of Marine Science, College of William and Mary, PO Box 1346, Gloucester Point, Virginia 23062

Although the use of triploid oysters in commercial production got its start in the mid 80's, it wasn't until 1994 that tetraploids were developed. Because of the highly original provenance of tetraploids, they were patented at Rutgers University and soon became the intellectual property of a private company for commercialization – the first breeding company for shellfish in the world. Arguably, this process of patenting the original technology slowed the rate of University research on tetraploids.

Commercial research and development on tetraploid brood stock began on the West coast of the US, followed by Australia, France and then the East coast of the US – these four areas represent the four major areas of commercialization so far. The first step in commercialization was creation of tetraploid populations that could be used for making triploids, by mating the tetraploid with diploids. Because genetic material cannot be readily transferred among regions, the exercise of tetraploid creation had to be initiated de novo in each area, and populations of putative and breeding tetraploids managed locally. Managing populations of tetraploids for the commercial hatchery is a sophisticated undertaking, requiring substantial infrastructure and in-house expertise.

Creation of tetraploids is followed by stabilization of tetraploid populations over time, a step that not only requires propagation of tetraploids by 4n x 4n matings, but for genetic management, should entail subsequent rounds of creation. Stable populations of tetraploids lead to stable commercial production of triploids, revealing some inherent traits of tetraploid oysters, including chromosome instability over time, variable phenotype and sex ratio, and difficulties with 4n x 4n propagation.

Further, triploids themselves were revealing some traits, such as, lower tolerance of stress in hatchery and field situations. Triploids clearly have economic advantages, however, and have gained partial popularity with oyster culturists for *C. gigas*, in most cases reaching about 50% of total production. An exception – triploids of *C. virginica* in the Chesapeake Bay (USA) have reached 80% popularity in only about five years, owing to multiple commercial advantages besides product quality.

Future breeding strategies for tetraploid oysters are just emerging. From the literature on breeding for plant polyploids, it seems the model is generally to improve diploids and subject them to polyploidization, which may be inappropriate for tetraploid oysters given their unique origin and the inability of clonal propagation. In fact, it is unclear whether breeding for the diploid, breeding for the tetraploid, or establishing a program of crossbreeding between diploid and tetraploids, with trait evaluation on the triploid – or some combination of both – is the most appropriate strategy. Approaches to improvement of tetraploids will be outlined, including some newly approaches to producing them.

The Sydney rock oyster *Saccostrea glomerata* as a biomonitor of estrogenic compounds

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Anthropogenic release of estrogenically active compounds is of widespread concern due to potential effects on reproductive development in aquatic organisms. An Australian native edible oyster species, *Saccostrea glomerata*, was chosen as a model organism to assess its utility as a bio-monitor for detecting effects of estrogenic compounds in Australian waters. Vitellogenin, precursor to the egg yolk protein, and accelerated female gonadal development are established biomarkers of endocrine disruption in fish and preliminary laboratory studies suggest they may be suitable as biomarkers for estrogenic exposure in molluscan models. To establish, if *S. glomerata* is a sensitive bio-indicator for detecting estrogenic compounds in Australian waters our project tested exposure of *S. glomerata* to estrogenic compounds under field conditions. The receiving waters of Burwood Beach wastewater treatment plant (NSW, Australia) were determined to be a suitable field location with estrogenic compounds and activity detected in effluent throughout the experimental period. Biomarkers, vitellogenin and mature female gonadal development, were elevated at Burwood Beach locations complementing laboratory findings and providing further evidence of the utility of *S. glomerata* as a biomonitor of estrogenic exposure.

Spatial and temporal distribution of norovirus and *E. coli* in oysters after a sewage overflow into a river.

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Human enteric virus contamination of oysters due to raw sewage contamination of oyster production areas is a problem worldwide. As a consequence of this, human illness outbreaks of norovirus (NoV) and hepatitis A virus (HAV) related to oyster consumption have occurred. Shellfish related outbreaks of gastroenteritis have occurred infrequently in Australia and the most recent documented shellfish related outbreak of NoV was in 2008 in New South Wales (NSW).

Oyster production areas in NSW are predominantly located in estuarine waters and these estuaries vary in size from large rivers to small lagoons and creeks. In locations where wastewater treatment plants discharge treated effluent into rivers, the river system can potentially act as a vector for the transport of viruses into oyster production areas. However, there is currently little information about the spatial and temporal distribution of NoV in oysters following the discharge of raw sewage into river systems.

To address this data gap, in 2010 we conducted a study on the spatial and temporal distribution of enteric bacteria and viruses in oysters following a raw sewage overflow into a river estuary production area. Sydney Rock Oysters, *Saccostrea glomerata*, were positioned at seven sites along a river, at a range of locations away from the potential source of pollution. The oysters were sampled at weekly intervals over a period of seven weeks following an overflow of 3000 kilolitres of raw sewage. Oysters were tested for NoV by Real-Time PCR and *E. coli* by a traditional 'mean probable number' culture-based method. NoV GII was detected up to 8.5 kilometres from the source of the raw sewage overflow over an extended period. As indicated by previous studies, the *E. coli* counts were elevated but did not consistently reflect the contamination of the oysters with NoV.

Rapid prediction of oyster biochemical composition using visible-near infrared reflectance spectroscopy (vnirs)

Malcolm Brown *, Stephen O'Connor and Matthew Cunningham

^{*}CSIRO Marine and Atmospheric Research, Food Futures Flagship, Australia

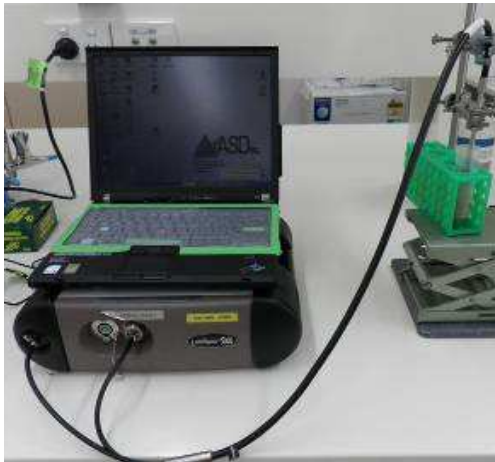
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Visible-near infrared reflectance spectroscopy (VNIRS) is widely used in the food and pharmaceutical industries for cost-effective, high-throughput analysis and quality control. The principle is that samples are illuminated by a spectrophotometer and the reflected VNIRS light (e.g. wavelength region 350 to 2500 nm) contains information that can be modelled by a computer to provide compositional data. Once the instrument has been calibrated with reference samples that have a known composition, it can measure multiple components simultaneously and rapidly.

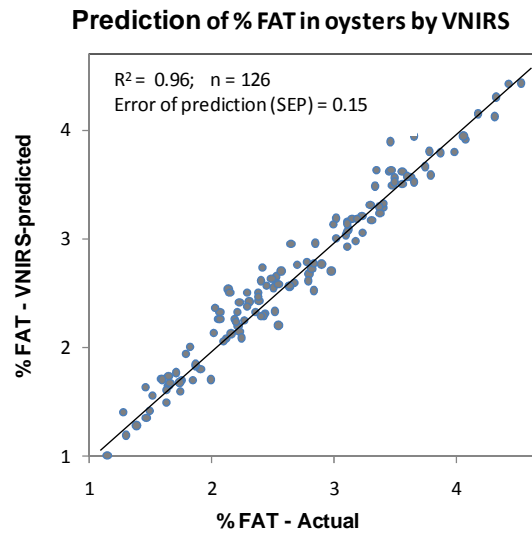
This study describes a novel application of VNIRS for the compositional analysis of oyster meat samples. Samples (*Crassostrea gigas* and *Saccostrea glomerata*) were individually homogenised, scanned by VNIRS (see photo below), subsamples chemically analysed, and calibration models developed to allow VNIRS-prediction. Comparison of predicted to actual (chemically measured) data showed excellent correlations ($R^2 \geq 0.92$) and low prediction errors for fat (see chart below), protein, glycogen and moisture. Preliminary results have also shown a good prediction for total omega-3 polyunsaturated fatty acids ($R^2 = 0.92$). These metrics indicate that the models are sufficiently accurate for quantitative applications. The key advantages of the methodology are: 1) its low

operational cost (once models have been developed) and 2) its high throughput, i.e. 250-300 samples can be analysed for all constituents each day. Therefore its use could be directed to applications requiring the rapid analysis of many individuals, e.g. in selective breeding programs where compositional data can provide valuable information on traits associated with animal condition or quality.

Three batches of oysters were also subjected to discriminant analysis. Models were developed that predicted the batch identity of the individual oysters on > 95% of occasions, providing a proof-of-concept that VNIRS may also have application in the qualitative analysis of oysters.



VNIRS system, with probe for scanning samples



Automation and grading equipment, why and whats next

Matthew Brown*

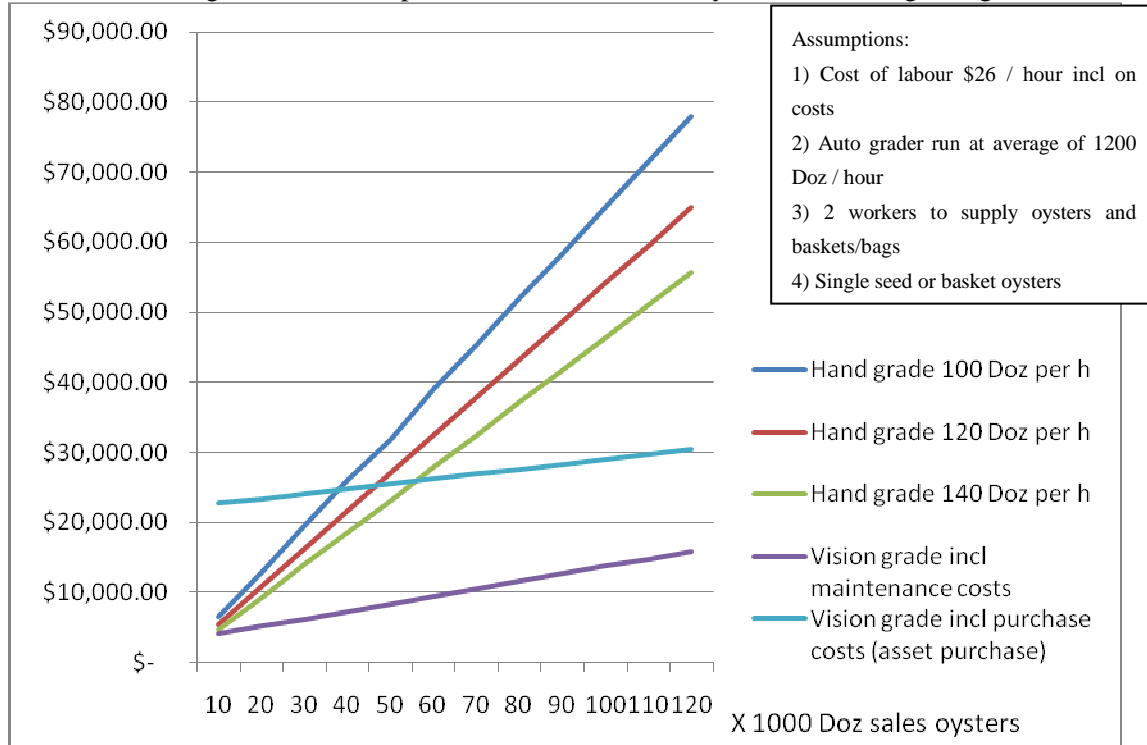
*S.E.D shellfish Equipment Pty Ltd, P.O.Box 415, Wynyard, TAS 7325 Ph: 61 3 6442 1563

Email: matthew@shellquip.com.au

- 1) Why invest in automation and what are the options?
- 2) Grading underwater why it's more accurate, cuts mortality and kills parasites.
- 3) How to cut the time and costs of re-bagging juvenile oysters!

Financial benefits of automated grading systems

The chart below gives a cost comparison between a vision system and hand grading



Example:

If a farm produces 60,000 Doz sale oysters per year and they can be hand graded at an average rate of 120 Doz per hour (red line) then the cost of that hand grading will be approximately \$35,000 per year.

For the same example using a vision grader and including the asset purchase repayment and maintenance. The cost comes to \$26,000 per year which means a saving of \$9,000 per year.

After 5 years and the vision grader has been payed off, the cost becomes \$9,000 and the saving per year \$26,000

Sensory and physicochemical assessment of *Crassostrea gigas*

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Australia's oyster industry aims to increase productivity without compromising quality. In addition, there is a desire to improve understanding of sensory properties of oysters to better communicate the diversity of oyster "tastes" to consumers, and also to guide decision making regarding growing variables. Studies have been conducted to understand oyster's quality from a conditioning point of view. However, sensory properties, and more precisely "taste", are known to be quality factors that will have the largest impact on consumer acceptance. This study aimed to develop a descriptive vocabulary for sensory evaluation oysters, and to establish relationships between sensory properties and oyster compositional data. *Crassostrea gigas* from 3 different regions: Tasmania, South Australia and New South Wales were harvested at the same time. A panel of 10 trained assessors evaluated oysters for aroma, flavour, texture and afterfeel using a standardized method of assessment and a consensus vocabulary of 12 descriptive terms. A sample of the same oysters was analysed by chemical methods to determine fat, protein, moisture, and glycogen content, and also free fatty acids (FFA) and free amino acids (FAA). Samples were also analysed using an NIR method.

Oysters were distinguished by earthy character, marine character, creaminess and saltiness. In addition, significant differences were found for firmness, chewiness, and juiciness. Regional differences were found, as were differences between individual oysters within region. For many sensory characteristics a bi-modal distribution was found, which was consistent for a number of correlated attributes. We believe the difference is due to seasonal variation based upon oyster gender. Significant differences between and within regions were also found for compositional data. In particular glycogen content was found to vary widely, as was the concentration of several FFA, and this variation was consistent with sensory character differences measured. The descriptive sensory method developed was successful in measuring eating quality of oysters and can now be applied more broadly.

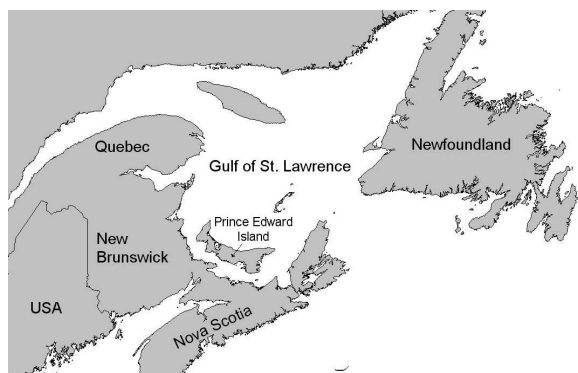
The effect of silt deposits on the spring awakening of eastern oysters in the Gulf of Saint Lawrence, Canada

Luc A. Comeau*, T. Jeffrey Davidson, Thomas Landry

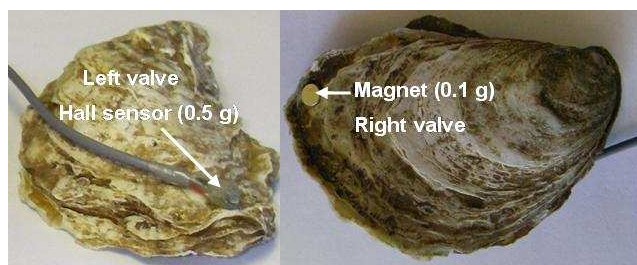
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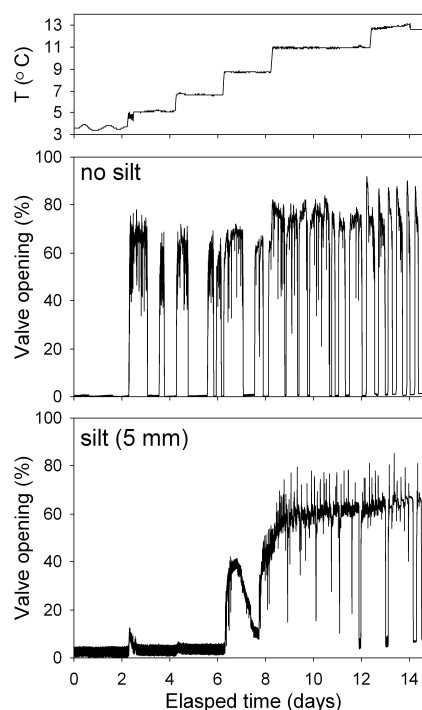
At their northernmost distribution limit in the Gulf of St. Lawrence, Canada, eastern oysters (*Crassostrea virginica*) are thought to be inactive for five consecutive months when the water temperature falls below 5°C. The lack of pumping activity over this extended period renders the natural and cultivated populations vulnerable to silt deposits. In this laboratory study, an innovative Hall sensor technology was used to closely monitor the valve movements of oysters buried in silt. Results corroborate the premise that valves are generally closed when water temperature is below 5°C. Above 5°C, oysters that were free of silt deposits exhibited a regular periodicity of valve activity. However, oysters buried under a 5 mm layer of silt exhibited stress responses as they attempted to reopen their valves and expulse the overlying silt. The atypical behaviours included a delayed "awakening" and a chronic display of low valve opening amplitudes. No mortality occurred over the course of the 15-day experiment. In a follow-up experiment, normal valve activity resumed after silt deposits were manually extracted from the holding tanks.



Map of study area in Atlantic Canada.



Oyster wired with Hall magnetic sensor assembly.



Time series showing the rising temperature in holding tanks, and valve opening amplitudes of a control (silt-free) oyster and a silted up oyster.

Cold shock control of Biofouling

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The Australian oyster industry has always encountered challenges from “over-catch” (fouling) from a myriad of sources including oysters, barnacles, mussels and sea squirts, flatworms, mudworm etc. While these pests are regionally specific, the issue is common across all growing areas and in all cases is a major financial burden.

Traditional oyster over-catch treatments have involved techniques such as extended periods of emersion, the application of salt or immersion in hot water (80°C). An alternative shown to have potential is cold shock treatment through immersion in saturated brine solution at temperatures of -16 to -20° C. In initial laboratory trials Heasman (2005) confirmed the potential of cold shock to treat Sydney rock oyster (*Saccostrea glomerata*) over-catch on large Pacific oysters (*Crassostrea gigas*).

To facilitate industry adoption of cold shock as a treatment for biofouling, trials have been undertaken to further assess cold tolerance across a broader size range of oysters and to assess its impact on other common fouling organisms.

Cold shock has been found to be particularly effective in treating “soft-bodied” pests such as flat worms (*Imogine mcgrathi*) and rapidly destroys smaller organisms such as barnacles (*Balanus* sp.) up to 1 cm in diameter. Cold tolerance of molluscs has been size dependent with small individuals succumbing faster. Comparatively, *S. glomerata* of up to commercial size are much less tolerant of cold shock than either *C. gigas* or Hairy mussels (*Trichomya hirsuta*) of a similar size.

Large scale trials are now underway to evaluate commercial-scale cold shock immersion baths in the field and to develop standard operating protocols for differing fouling types.

Oyster larvae are in deep trouble at high-CO₂ in South China: results of a long-term and large-scale experiment

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Oysters are commercially important coastal species and have a complex life cycle, during which the swimming (pelagic) larvae must select a suitable substrate, attach to it, and then metamorphose into benthic adults. Natural environmental change and anthropogenic activities, i.e. rising CO₂, have resulted in a fluctuating and variable carbonate chemistry or ocean acidification (OA), which has the potential to greatly influence the success of this key metamorphic transition by potentially affecting both appropriate shell biomineralization events and physiology. Calcifying larval species may suffer more in this century as carbonate ions continue to decrease and the critical question of how this rising CO₂ might affect their key developmental, physiological, biomineralization and molecular processes remains largely unaddressed. Recently, a few larval biologists, in collaboration with molecular biologists and material scientists, have begun to address this question. Our large-scale and long-term controlled experiment at commercial hatchery setting showed that larval shell growth and importantly their settlement and recruitment rates are significantly reduced at projected carbonate chemistry scenarios in 2100 to 2300 in the oyster species (*Crassostrea hongkongensis*) that support the livelihoods of millions of people in South China

Progress, transitions and challenges in the Sydney rock oyster breeding program

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The Sydney rock oyster, *Saccostrea glomerata*, breeding program began in 1990 with the aim of developing faster growing, winter mortality resistant oysters to increase industry profitability. Using mass selection the program has successfully done this as well as expanding in 1997 to also produce lines that are resistant to a second disease that has devastated the industry called QX (Queensland unknown). Disease resistance breeding has taken place in the Georges River, Sydney as this estuary is affected by both winter mortality and QX disease and selection for breeding has predominately been based on survival and growth since the program commenced.

Novel production technologies developed in the last four years enabled the breeding program to significantly expand to include pedigreed pair-mated families. In 2007, 31 families were created to investigate the role of the phenoloxidase enzyme cascade in QX disease resistance. In the following year, a further 27 families were created by within-line crosses of the Georges River mass selected lines producing families resistant to QX disease, winter mortality disease and both diseases. Resistance of these families was assessed by rotating oysters between sites affected by each disease in the Georges River. Since this date, a further 30 families have been created from wild (non-selected) Sydney rock oyster crosses. More recently, monitoring of additional performance criteria related to meat condition and shell shape have been included for each family and mass selected line as differences in reproductive status between wild and fast growth oysters has been found.

In November 2010, an outbreak of ostreid herpesvirus (OsHV) occurred in the wild and farmed populations of Pacific oysters, *Crassostrea gigas*, in the Georges River. No losses of Sydney rock oysters were observed, however OsHV was detected by PCR in Sydney rock oysters including samples collected from broodstock used in the breeding program. This disease outbreak has raised a

number of logistical challenges for the breeding program to overcome in the future related to accessing broodstock and progeny confined to this estuary and identifying appropriate alternate estuaries that can be used to continue to improve disease resistance and other key traits among Sydney rock oyster families and mass selected lines.

SWOT analysis - Innovative approach to sustainable oyster culture in Vietnam

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The recent success of producing oyster seed recently enhances the production of Pacific oyster and expands its industry in Vietnam. A SWOT analysis would help to search for strategies to achieve a sustainable oyster industry. The strengths of oyster industry are the favourable environment for oyster growth and low labour cost. However, the less advance technology limits the development of this industry. Because of lack of oyster processor and promotion for oyster products, and less diversified products, the oyster demand is oversupply. Oyster products have not yet been under quality control or inspection. Despite several weaknesses, the opportunities for local and international collaboration and companies to invest in any stage from spat to table are available. More research needs to be done to prevent the threads of reducing growth or killing oyster such as industrial competition, climate change as well as disease, and of high competitive market.

Genetic immunity and disease resistance in Sydney rock oysters

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Mass mortalities of farmed Sydney rock oyster, *Saccostrea glomerata*, have been observed in Australia since the 1970s due to the paramyxean protozoan parasite, *Marteilia sydneyi* (aetiological agent of QX disease). Identification of the genes involved in resistance of *S. glomerata* to *M. sydneyi* and their DNA variants is critical for marker-assisted selection of QX-resistant oysters. Recently, we showed that the base-line expression of the peroxiredoxin 6 (Prx6) gene is significantly lower in *S. glomerata* bred for QX-resistance than non-selected control oysters. The expression of Prx6 is highly regulated in *S. glomerata*. Injection of a range of different pathogen associated molecular patterns (PAMPs) failed to induce the expression of Prx6, but changes in the expression of Prx6 was observed when oysters were exposed to environmental stresses, such as salinity (known risk factor for QX disease). In-situ hybridization using a Prx6 specific probe revealed that the sub-populations of hemocytes that express Prx6 is equal between QX-resistant and -susceptible oysters. Therefore, the differential expression of Prx6 is likely to originate from modifications of either mRNA stability or gene transcriptional rate.

In an attempt to understand why the base-line expression of the Prx6 gene is down-regulated in QX-resistant oysters, polymorphisms within the *S. glomerata* Prx6 gene and 5 upstream region were identified and the frequency of these polymorphisms between QX-resistant and -susceptible *S. glomerata* determined. A total of three indels and 11 single nucleotide polymorphisms (SNPs) were identified in a 614-bp fragment of the Prx6 promoter, some of which potentially affect the binding of several regulatory elements. Notably, the frequency of a SNP at position -240A>G. The genotypic frequency of -240G/G was 0.400 in resistant oysters compared to 0.067 in susceptible oysters ($p = 0.059$). The presence of -240G/G resulted in the absence of a putative heat shock element in the Prx6 promoter region in position -238 to -242. The potential of this SNP as a marker for disease resistance in *S. glomerata* will be discussed.

Molecular pathways involved in the gametogenesis of Pacific oysters *Crassostrea gigas*

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The Pacific oyster *Crassostrea gigas* is marine bivalve of major economic and ecological importance. *C. gigas* is an alternative hermaphrodite and presents a massive and very flexible allocation to reproduction that makes an interesting model to study factors affecting reproductive effort and trade-offs with other fitness-related traits. Moreover, oysters have a very high fecundity that contributes to their invasiveness in an increasing number of countries. Conversely, reproductive effort might have a negative impact on cultured stocks suggested by negative phenotypic and genetic relationships between reproductive effort and summer survival. In this context, studies on the physiology, genetics and genomics of reproductive traits in *C. gigas* are important to optimize aquaculture production and to understand how and why it can turn into an invasive species.

In the Ifremer's Laboratory of Invertebrate Physiology (Plouzané, France), one of our main focus is to study physiology of reproduction in marine bivalve species and especially in the Pacific oyster. To achieve this objective, we follow two kinds of approaches: first, global analyses are used to elucidate molecular signals for reproductive traits by identifying genes and proteins that are specifically or preferentially expressed at each reproductive stage; secondly, specific analyses of some genes and proteins are used to characterize the links of energetic metabolic pathways with gametogenesis and energy allocation devoted to reproductive effort.

From a whole genomic approach in oysters and/or information from model organisms, we identified markers specific to reproduction and thus developed functional approaches to unravel the role of these candidates in the Pacific oyster. Among them, we identified AMP-activated Protein Kinase (AMPK), an evolutionarily conserved protein kinase complex that acts as a fuel gauge in regulating energy metabolism in many species. The AMPK system is an energy balance regulator that, once activated by low energy status stimulates ATP-producing catabolic pathways and inhibits ATP-consuming anabolic pathways. The aim of my thesis is to characterize the function of AMPK signaling pathway in *C. gigas*. First, we identified specific sequences of AMPK subunits and assayed the spatio-temporal expression of this complex enzymatic system. Then an AMPK pharmacological stimulator (AICAR) has been used to analyse the role of AMPK activity through the win-of-function phenotypic identification. Furthermore, to complete this picture of AMPK network in *C. gigas*, identification of targets of AMPK will be performed using a phosphoproteome scanning of the gonad before and after pharmacological stimulation.

Effect of the food containing oyster extract on stress, fatigue and quality of sleep in working persons

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An open-label study was conducted to evaluate the effect of food tablets containing oyster extract as a major ingredient (test food), 12 tablets/day for 8 weeks, on the stress level, sleep quality, fatigue, and quality of life in 17 adult male and female workers who were aware of fatigue and sleep disorders in their daily life, using subjective evaluation results and markers in the blood and saliva as indicators. The study results are summarized as follows: Regarding stress and fatigue, T-scores of negative moods, such as tension-anxiety (T-A), fatigue (F), confusion (C), anger-hostility (A-H), and depression (D), by the Japanese version of the Profile of Mood States (POMS) short form, were significantly improved at 1 to 2 weeks after consumption of the test food. Four weeks after consumption, the T score of vigor (V), a positive mood, was also significantly improved. Regarding sleep quality, scores of the OSA Sleep Questionnaire and Pittsburgh Sleep Quality Index were significantly improved at 2 to 6 weeks after consumption. Regarding biological markers, salivary

cortisol level immediately after rising on working days gradually increased, and significantly increased at 8 weeks after consumption. The difference between the salivary cortisol level immediately after rising and at 30 minutes after rising (CAR) gradually decreased compared to the difference before consumption, and significantly decreased at 8 weeks after consumption. The serum level of selenium, a trace mineral, significantly increased at 8 weeks after consumption. Regarding the quality of life (QOL), subscores of sleep were improved at 2 to 8 weeks after consumption.

These results showed that consumption of food containing oyster extract for 8 weeks was effective in improving subjective sleep quality and negative psychological state, reducing fatigue, and slightly improving stress response of the body (awakening cortisol response) and quality of life, in subjects who were aware of fatigue and stress caused by sleep problems in their daily life. Regarding safety, clinically significant subjective symptoms or changes in measured values were not observed.

Pacific oysters (*Crassostrea gigas*) from different family lines demonstrate different susceptibility to infection with Osterid herpesvirus-I after natural challenge

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The recently described microvariant of Osterid herpesvirus-I (OsHV-1 μ var) has caused disease outbreaks with high mortality in Pacific oysters in the several countries in Europe over the previous 3 years. In late 2010 outbreaks were also confirmed in Australia and New Zealand. Management of this emerging disease is a global priority based on the devastating effects on the oyster production industry in France. In Australia, OsHV-1 μ var was identified as the cause of a disease outbreak in the Georges River, south of Sydney, in November 2010. This disease outbreak resulted in >98% mortality of farmed Pacific oysters and a high mortality was also observed in wild Pacific oysters. The disease investigation included ongoing monitoring of oyster populations in the Georges River using a real-time polymerase chain reaction (qPCR) assay which enabled rapid and sensitive detection and quantification of OsHV-1. The prevalence of infection in the few surviving farmed Pacific oysters in June was 95% (n=37), 7 months after the disease outbreak. At this time the prevalence of infection was lower (25%) in wild spat recruited after the outbreak (p<0.01). High mortality and 100% prevalence of OsHV-1 infection occurred over a 2 week period when sentinel Pacific oysters were translocated into this waterway 3 months after the start of the outbreak (n=42).

A pilot trial was subsequently initiated to determine if there was any difference in the susceptibility of farmed Pacific oysters of different genotypes. Pacific oyster broodstock from 20 different family lines were subjected to a natural OsHV-1 challenge by translocation into the Georges River, close to the location of the disease outbreak. There were significant differences in the prevalence of infection (range 3% – 95%; p<0.01) in different family lines. There were also marked differences in mortality between some family groups (range, 0 – 27%; n=30), with 4.5% cumulative mortality for the entire group (n=600). A combination of host and environmental factors are thought to influence the occurrence of disease associated with OsHV-1 infection. As this trial was undertaken at a time when water temperatures were declining, different infection and mortality rates may be detected during warmer weather. Nevertheless, these promising results suggest a probable genetic basis for resistance to infection with OsHV-1. Further work is required to investigate the possibility of producing disease resistant seedstock which will allow continued production in the presence of this emerging disease threat.

Sequence polymorphism from mitochondrial noncoding region of the Portuguese oyster *Crassostrea angulata* in Taiwan

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Portuguese oysters (*Crassostrea angulata*) are an important marine resource within the seashores of Taiwan. It has been mistakenly recognized that Portuguese oysters are Pacific oysters in Taiwan. Nevertheless, Portuguese oyster is the most consumed oyster in Taiwan. Even though several studies have been conducted on the farming methods and culturing techniques, its genetic population structure has never been investigated. Data on genetic structure of Portuguese oyster populations is essential in determining the impact of human activity on the overall genetic variability. It can also be applied to estimate the populations through transportation of individuals from different locations. To evaluate genetic variability in Taiwan, we were using mitochondrial DNA noncoding region (between tRNA Glycine and tRNA Valine) from 188 individuals within the Taiwan coast area. 117 variable sites were detected in the 652 bp noncoding sequences and 123 haplotypes were defined. We have compared the result with *Crassostrea gigas*, and it revealed the *Crassostrea angulata* collected from Taiwan has much highly polymorphism. The result also supported The *Crassostrea angulata* origin from Taiwan.



Figure 1. The oyster farm in Taiwan. Farmers collect oysters from ropes.

Genetic diversity of Pacific oyster in Japan

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Pacific oyster *Crassostrea gigas* is now the most widely consumed and commercially important oyster in the world, owing to its long historical introduction from Japan to Australia, Europe, and the Americas. A framework for genetic conservation of wild stocks of *C. gigas* in Japan is thus a

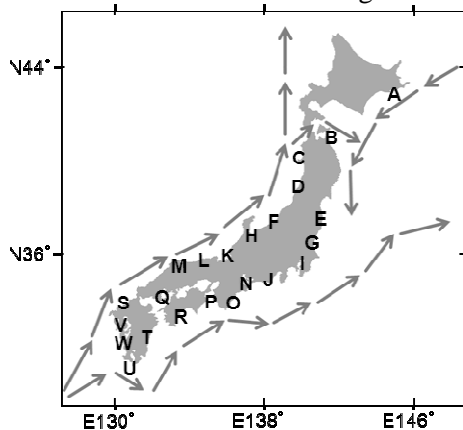


Fig. 1. Map of Japan showing sites A to W at which 1,079 individuals were collected. Arrows indicate the approximate routes of the

prerequisite for its sustainable global production. However, the status of its genetic structure and diversity in Japan has so far been poorly understood. In this study, nucleotide sequence analysis of the mitochondrial DNA region (507 bp) encoding the COI gene was conducted to elucidate population genetic structure of *C. gigas* throughout Japan. A total of 128 haplotypes were observed on the basis of 107 variable nucleotides among 1,079 individuals collected from 23 sites in Japan (Fig. 1). The haplotype-001 was dominant in all 23 populations, and the parsimony network tree showed the radiation from the focal haplotype-001 to other 127 haplotypes with 1-4 nucleotide substitutions, suggesting shallow haplotype genealogy of *C. gigas* in Japan. Nucleotide diversity and haplotype diversity values were calculated to be 0.130 % and 0.451 among all individuals, and ranged from 0.072 % (site N) to 0.259 % (site R) and from 0.257 (site N) to 0.663 (site R) within

sites, respectively. These genetic diversity parameters depended on the observed haplotype numbers and frequency of the dominant haplotype, because high number of haplotypes and relatively low frequency of the haplotype-001 appeared in site R, as compared with other sites. All pairwise F_{ST} values between sites were calculated to be less than 0.027, and this generally low level reflects small genetic differentiation caused by high gene flow of *C. gigas* in Japan. Meanwhile, mismatch distributions analysis for sites R, S, T, and V and other 19 sites were determined as multimodal and unimodal statuses, respectively. In addition, the average numbers of pairwise nucleotide differences within sites R, S, T, V plus U and W, all of which were located in southwestern Japan, were higher than those within other 17 sites. This pattern of genetic diversity could be attributed to unidirectional northeastward migration and subsequent population expansion of *C. gigas* from southwestern region to other regions probably by the ocean currents around Japan and/or global environmental changes during the Quaternary glacial-interglacial cycle. Incidentally, Kimura's two-parameter distance of pairwise divergence of haplotypes was calculated to be 0.550 %, which corresponded to ca. 0.10-0.21 million years ago based on 2.630-5.260 % per million years for *C. gigas* and closely related *C. angulata* according to O'Foighil et al. (1998). The sea level had fallen and risen during the Riss glacial and subsequent interglacial stages from ca. 0.25 to 0.07 million years ago in the Middle-Upper Pleistocene.

An abnormal enlargement of the ovary in the Pacific oyster – an old and recurring problem in oyster culture industries of Japan

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In Japan, annual aquaculture production of bivalves reaches 81 million tons, and Pacific oyster is one of the most important aquaculture species. Although summer mortality events caused by high water temperature are occasionally reported, so far, no serious lethal diseases for oysters have recognized in



Fig. 1. Pacific oyster with abnormal enlargement of ovary.

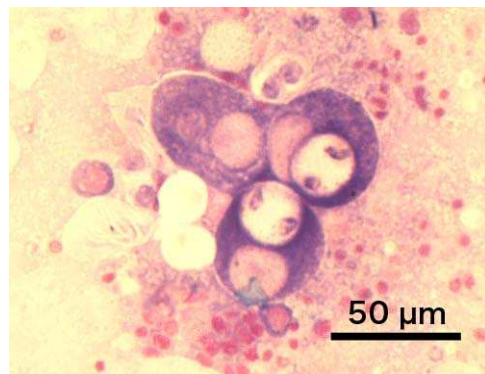


Fig. 2. *Marteilioides chungmuensis* in egg cells of Pacific oyster.

Japan. On the other hand, oyster industries, particularly in the western part of Japan, have suffered from an enigmatic disease without death of oysters - abnormal enlargement of the ovary in oysters. Diseased oysters show anesthetic appearance with nodule-like structures in the ovary, resulting in the loss of its marketability (Fig 1).

Abnormal enlargement of ovary in oysters was first reported in 1934 as a physiological disorder, and currently it is known that this disease is caused by a paramyxean protozoa, *Marteilioides chungmuensis* (Fig. 2). Although this disease is one of the oldest known oyster diseases in the world, little biological information on the parasite has been accumulated, and no countermeasures to the disease have been established.

Recently, the small subunit ribosomal RNA sequence of *M. chungmuensis* was identified, and since then various biological aspects of this parasite were rapidly elucidated by molecular studies. Additionally, intensive field research recently suggested a countermeasure, currently in the experimental phase.

In this presentation, we would like to introduce current research topics on this diseases, and "recent events" which shed light on this disease in Japan.

Phenoloxidase phenotypes are associated with mortality in families of Sydney rock oysters produced by single-pair mating

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The defensive enzyme, phenoloxidase (PO), has previously been linked to disease susceptibility in Sydney rock oysters (*Saccostrea glomerata*). In mass selected *S. glomerata* populations, the expression of distinct electrophoretic phenotypes of PO is strongly correlated with susceptibility to QX disease. However, previous studies have not taken into account the hereditary background of QX disease resistant and susceptible populations. To negate the non-specific effects of mass selection, the current study compares the frequencies of different PO phenotypes with mortality among oyster families produced by single-pair mating.

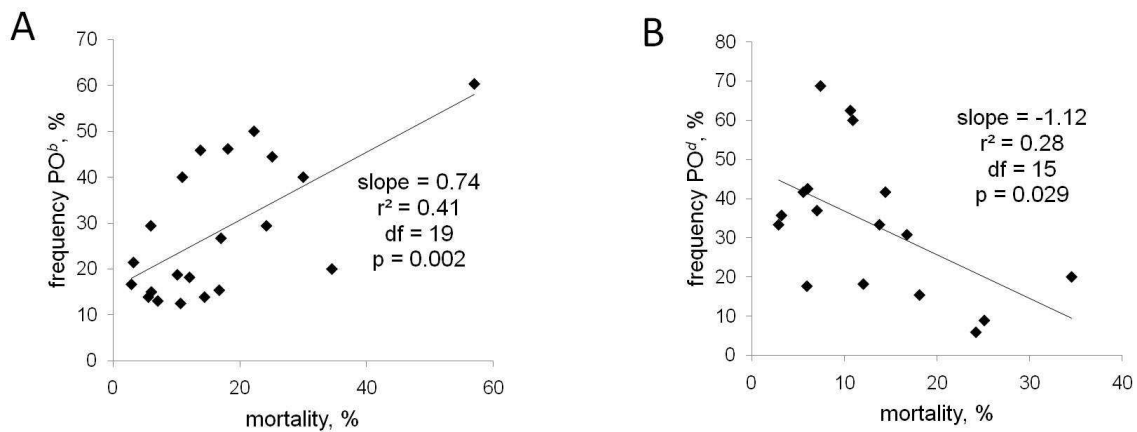


Figure 1. Linear regression analysis of mortality vs. the frequencies of two different forms of PO (A. PO^b; B. PO^d) in single-pair mated families of *S. glomerata*. Each point is data for one single-pair mated family.

Five different forms of PO were identified by native polyacrylamide gel electrophoresis in single pair families bred from QX disease resistant parents. The resulting data corroborate previous findings from mass selected lines of *S. glomerata*. In single pair families, one form of PO (PO^b) was positively correlated with mortality, whilst another (PO^d) was negatively correlated with mortality (Figure 1). In contrast, there was no relationship between mortality and another enzyme that has been implicated in disease resistance, superoxide dismutase (SOD). These results strengthen the association between PO phenotypes and mortality associated with QX disease, but suggests that other genetic factors also contribute to survival. Comparisons between the PO phenotypes of parents and their offspring also indicated that the different electrophoretic forms of PO are not simple alleles at a single PO gene locus.

Edible oyster culture in tropical Australia

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Globally, tropical oysters are widespread and are often harvested from wild populations. In contrast, tropical oyster culture is limited to a few countries only such as Mexico, Vietnam and the Philippines. In Australia, the black-lip oyster *Striostrea mytiloides* and the milky oyster *Saccostrea cucullata* have been harvested from the wild since pre-historic times.

Experimental trials to culture *S. mytiloides* have met with success in Queensland but this has not led to the establishment of a tropical oyster industry. Here we report on the development of hatchery, nursery and grow-out protocols to farm *S. mytiloides* in the Northern Territory (NT). An overview of the aims and challenges is presented.

The effect of environmental stress on the Pacific oyster *Crassostrea gigas*: freshwater flooding and the effects of abrupt low salinity

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Over time climate patterns are predicted to change and these changes may increase the frequency of environmental stressors, such as freshwater flooding in summer, for oysters farmed in estuaries around Tasmania. Understanding how oysters respond to environmental stressors can help farmers minimise stock losses, by changing management or adapting farming practices. Histopathology is a useful tool to study the mechanism of oyster response to environmental stressors and disease. During fresh water flooding there are multiple risk factors for oyster mortality including abrupt low salinity, eutrophication, decreased dissolved oxygen, and acid sulphate soil run-off.

This work describes a retrospective histopathology study of Pacific oysters sampled over a three year period from the northeast coast of Tasmania. Oysters were sampled from a fresh water flood mortality event in February 2004, in Georges Bay / Moulting Bay. These oysters showed microscopic osmotic changes, which correlated to the abrupt fall in salinity during the flood. These changes included expanded interstitium under the mantle, expanded intercellular spaces in the gastric, intestinal and digestive tubular walls (accompanied by intramural haemocyte infiltrate) ($P < 0.001$) and dilated renal tubules with expanded intercellular spaces and intracellular vacuolation ($P < 0.001$). Additional microscopic changes (not consistent with osmotic changes) included dilated digestive glands and necrosis of leydig cells (suggestive of decreased feeding / closed shell / metabolic catabolism) ($P < 0.001$) and multifocal erosion of the mantle ($P < 0.001$). This range of microscopic changes was not seen in oysters sampled in February 2003 and October-November 2005 from the same region, when there was no flooding.

Subsequent tank trials demonstrated oysters in summer, rather than winter, had similar osmotic related microscopic changes following low salinity stress ($P < 0.001$) compared to control oysters at normal salinity. Whilst these reversible osmotic changes were not suspected as being the primary mechanism of mortalities during the February 2004 flood event they could have compromised host innate immune response / alimentary barriers and predisposed to mortalities through other flood induced factors.

Further research will clarify how other factors in fresh water floods interact with low salinity on Pacific oysters. Future research will investigate how low salinity compromises cellular functions (gene expression) and immune competence.

Pacific oyster selective breeding in Australia: the past present and future

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Selective breeding for Pacific oysters (*Crassostrea gigas*) began in Australia in 1997. Although applied breeding has a long history in terrestrial animal production, it was at that time relatively novel to shellfish industries. Consequently, this breeding program has been required to continually address new challenges and evolve to face new situations. This has included addressing technical issues and adapting to different commercial situations. The evolution of the ASI oyster breeding program will be outlined, including a summary of the main issues that have been addressed, progress that has been made, and issues that are currently in the process of being addressed. Adapting to the future is likely

to require continued change and adaptation, and perhaps more so than the past. Changes to environments and markets are predicted, and changes to genetic technologies are certain. Opportunities for new developments in applied breeding will be outlined, and the relative merits of these will be discussed with specific reference to applied oyster breeding.

Noradrenaline induces apoptosis of akoya pearl oyster haemocytes

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Fluctuating environmental conditions can lead to physiological stress and up-regulation of stress-associated hormones in bivalves. These hormones include noradrenaline (NA). Environmental stress has also been found to suppress immune responses in the Akoya pearl oyster, *Pinctada imbricata*, primarily by impairing the activity of defensive hemocytes. In the current study, we investigated the *in vitro* affects of NA exposure on *P. imbricata* haemocytes, particularly the ability of NA to induce hemocyte apoptosis that could lead to immunosuppression.

Terminal dUTP nick-end (TUNEL) labelling (a late stage apoptotic marker) was used to detect cells displaying DNA fragmentation within tissue exposed to NA. DNA fragmentation was significantly increased when cells were exposed to 10.0 ng NA/ μ g relative to non-treated controls ($p < 0.05$). Similarly, annexin V-FITC staining (a marker of early apoptotic events) was evident in cells exposed to 5.0 and 10.0 ng NA/ μ g after 120 min ($p < 0.05$). Haemocyte adhesion to glass slides and their capacity for filopodia formation also declined significantly when cells were exposed to 10.0 ng NA/ μ g ($p < 0.05$).

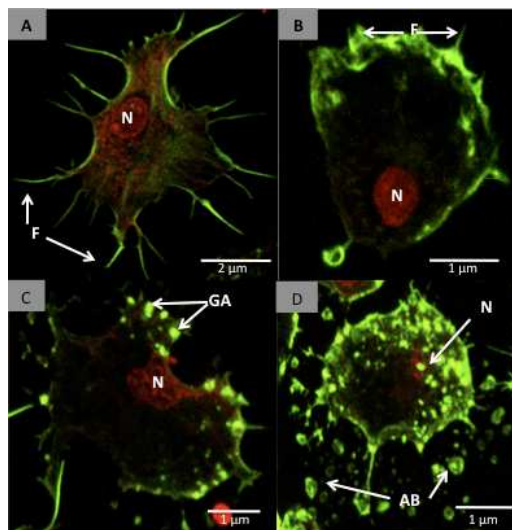


Figure 1. Confocal fluorescent micrographs of control and NA-treated haemocytes stained for F-actin with phalloidin Alexa Fluor 488 (green). Nuclei (N) are counterstained with propidium iodide (red). A Control (non-exposed) haemocyte. B, Haemocyte exposed to 2.5 ng NA/ μ g for 10 min. C, Haemocyte exposed to 5 ng NA/ μ g for 30 min. D, Haemocyte exposed to 10 ng NA/ μ g for 30 min. GA; globular actin, AB; apoptotic body; F, filopodia.

A number of morphological and ultrastructural changes were also identified in NA-exposed haemocytes using transmission and scanning electron microscopy. These alterations included chromatin and cytoplasmic condensation, the formation of apoptotic bodies, vacuolization and blebbing. In NA-treated cells, there was substantial remodelling of the actin cytoskeleton and polymerization of F-actin was observed around the periphery of the cytoplasm (Figure 1). These data suggest that NA induces apoptosis in *P. imbricata* haemocytes, and that cytoskeletal alterations during the apoptotic process may impair immune functions such as phagocytosis.

Genetics and breeding of the Pacific oysters in China: Progress and prospects

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To improve yields of the Pacific oysters (*Crassostrea gigas*), a one-generation selection was performed in the Pacific oyster using three stocks from China (Stock C), Japan (Stock J) and Korea (Stock K) in 2007. Applying about the same intensity of selection in the upward direction, three selected (CS1, JS1, and KS1) and three control lines were created, which were reared under the same environmental conditions at larvae, spat, and grow-out stages. Stock C and Stock J showed significantly higher response to selection and realized heritability than Stock K at spat and grow-out stages ($P < 0.05$). At harvest on day 360, the selected lines of Stock C, J and K grew 12.2%, 12.2% and 7.9% larger than their control lines on shell height, respectively. When averaged crossing grow-out, the genetic gain for Stock C, J and K was $13.2 \pm 1.2\%$, $13.2 \pm 1.0\%$, and $7.2 \pm 0.7\%$, respectively; and realized heritability for Stock C, J and K was 0.334 ± 0.028 , 0.402 ± 0.024 and 0.149 ± 0.027 , respectively. The relatively high realized heritability estimate obtained from Stock C and Stock J indicates that there is genetic variation in the two stocks and that selective breeding by mass selection is very promising.

To determine whether continuous progress can be achieved, a second-generation selection was conducted in the three breeding lines in 2008. The progeny of three second generation Pacific oyster lines were evaluated in a 400-day farming experiment. At harvest on day 400, the selected crosses of the CS1, JS1, and KS1 lines grew 9.1%, 10.2% and 9.7% larger than the control crosses. During grow-out stage, the genetic gain for the CS1, JS1 and KS1 lines was $10.1 \pm 1.4\%$, $10.4 \pm 0.3\%$, and $8.5 \pm 1.7\%$, respectively; and realized heritability for the CS1, JS1 and KS1 lines was 0.443 ± 0.139 , 0.344 ± 0.077 and 0.369 ± 0.010 , respectively. Selection for fast growth achieved steady progress in the second generation. These results provided encouragement for the continuation of selective breeding program in the Pacific oyster in China.

Modified atmosphere packaging of half shell Pacific oysters *Crassostrea gigas*

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Oysters are a highly nutritious food source as they provide an abundance of important minerals and possess a fatty acid profile dominated by beneficial polyunsaturated fatty acids. Oysters sold at retail in Australia are generally presented as a half shell product. The Australian oyster industry has identified retail sales into supermarkets as an opportunity for increased sales. However, retailers demand that products be innovative and utilise packaging strategies that result in increased shelf-life and convenience without impacting on safety.

Modified atmosphere packaging (MAP) refers to the manipulation of gases within an enclosed chamber to extend the shelf-life of foods contained within the chamber. MAP is currently used to extend the shelf-life of fresh meat, fruits and vegetables. Three gases are predominantly used: carbon dioxide (CO₂) oxygen (O₂) and nitrogen (N₂). CO₂ inhibits the growth of aerobic microorganisms and limiting O₂ can reduce oxidation of product, whilst nitrogen is used as an inert filling gas. However, each product type must be assessed to ascertain the most suitable mixture and concentration of gases to extend shelf-life.

MAP technologies have been applied to many types of seafood products including finfish, crustacea and bivalve molluscs. However, to date, no studies have been reported that evaluate the effects of a wide range of MAP treatments on either whole shell or half shell oysters. This work aims to establish

if MAP can extend the organoleptic shelf-life of half shell oysters and identify the most appropriate atmospheric mix for half shell oysters. Natural processing aids are also evaluated to establish if they can further extend shelf-life.

Oysters were shucked as per industry practice and sealed in plastic trays with a barrier film using a VC999 tray sealer. Various atmospheric mixes were compared against a standard air atmosphere control. Oysters were held at 4°C until spoiled and assessed periodically for total viable counts, counts of lactic acid bacteria and *Shewanella*-like bacteria. Sensory analysis was also undertaken to assess odour of oysters and opacity of shell liquor. The concentration of trimethylamine, a compound that is often responsible for off odours in spoiled seafood, was also determined. The most effective atmospheric mix was then selected for a further experiment to assess the usefulness of both an essential oil-based and a chlorine dioxide-based processing aid in comparison to a control group processed using a normal washing process.

Results of these experiments will be presented and the findings discussed with reference to the potential for prolonging shelf-life of oysters. This extension of shelf-life may allow industry increased access into other markets such as retail environments.

Larvae culture experiment on mangrove oyster *Saccostrea cucullata* at Bali, Indonesia

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Large size of oyster *Saccostrea cucullata*, a tropical species with a shell length more than 10 cm, are found in the mangrove forests in Batu Ampar Bay located in the northern part of Bali, Indonesia. To obtain the technical knowledge of aquaculture of this species, experiments on larvae culture were begun since 2009. Larvae were cultured in our hatchery in Gondol, Bali, base on the "Hatchery culture on bivalves (FAO)" as feeding three kinds of phytoplankton, *Chaetoceros calcitrans*, *Isochrysis galbana* and *Pavlova lutheri*. Various experiments being carried out, whereas no metamorphosis of larva have been observed up to present. Larvae reached to ages 10 to 14 days with a maximum shell length 148 µm, but the larval mortality gradually increased during this period, no larva finally survive beyond 14th day for each experiment. To make clear the reason and to prevent the mortality increase during this critical period, some improvements of rearing water quality for larvae and of conditioning by feeding for blood stocks are continued.

Clinical efficacy of Pacific oyster extract on sperm profiles in healthy male subjects

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In recent years, WHO reported shocking phenomena that sperm motility function has decreased by half since last 50 years. The cause of the phenomena is complicated and unclear, however, WHO pointed out stress, environment pollution, poor nutrition and so on. In addition, there is a report which suggests relationship between depression of the sperm motility and lack of trace elements such as selenium and zinc. To examine the effect of the extract from the soft-body sites of *Crassostrea gigas*, that is enriched with trace metals such as zinc, on low sperm profiles, we used "Watanabe active oyster", a test food that mostly composed of the extract. The test food was administered to healthy subjects with the low concentration of sperm (standard level, $>2 \times 10^7/\text{ml}$; subjects, $1.3 \times 10^7/\text{ml}$) and the low concentration of progressive motile sperm (standard level, $>5 \times 10^6/\text{ml}$; subjects, $1 \times 10^6/\text{ml}$) (12 tablets per day for 8 weeks). An inclusion criterion was set for the sperm motility with less than or equal to 60%. Both sperm concentrations were significantly increased to the standard levels 8 weeks after administration. The concentrations of zinc and selenium in the serum,

which are within the standard ranges, were not changed. These results indicate that the test food has the ameliorative effects against the low sperm concentrations and sperm motility function that are closely associated with fertilization.

Development of tools for the sustainable management of genetics in polyploid Pacific oysters (*Crassostrea gigas*)

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Triploid Pacific Oysters (*Crassostrea gigas*) are an important part of commercial oyster production throughout the world. Despite this, many features of polyploid oyster biology still remain uncertain. In particular, ways to manage genetic diversity and inbreeding in polyploid oysters populations are unknown. To understand current diversity levels and provide a baseline for what diversity is available, a large population genetic study was completed on native, naturalised and cultured diploid Pacific Oysters. Ten polymorphic microsatellite loci were multiplexed to analyse a total of 368 Pacific Oysters (*Crassostrea gigas*) sampled from native (Japan and Korea), naturalised (France and Australia) and cultured (three Australian programs) populations. Results based on sample location and Bayesian analysis both indicated a high level of diversity with only a small reduction in variation within the cultured samples. No difference was observed between the native and naturalised samples suggesting that this species has lost little if any diversity since its introduction. The results indicate that breeding techniques within Australian oyster hatcheries are likely adequate for maintaining diversity. The second stage of this study involved comparing tetraploid diversity to that of its diploid counterparts. It was found that within two tetraploid lines (86 individuals in total), the average number of alleles (12.7) were slightly lower than the diploid populations (Cultured = 16.6, Wild = 29.2). Average heterozygosity, however, was higher in the tetraploid population (0.83) compared to the cultured diploid populations (0.81) but still lower than the wild populations (0.89). The results indicate that breeding techniques for tetraploid management are likely adequate for maintaining diversity. The microsatellite markers confirmed the individuals were tetraploids with half of the loci having three or four alleles in over 70% of the individuals. The efficiency of these markers to assign pedigree in a tetraploid population is being evaluated.

An industry led approach to managing risk: developing EMS in the NSW oyster industry

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The future of the oyster industry depends on our capacity to demonstrate that natural resources and the environment in which we work, are utilised in a sustainable, responsible way. One way of doing this is to develop an Environmental Management System (EMS) to demonstrate how environmental impacts and risks are managed.

OceanWatch Australia, in partnership with the NSW Farmers Oyster Committee, are working with groups of NSW oyster farmers to develop, review and implement EMS across the industry. With voluntary participation, engaging in the EMS process offers many benefits, including the opportunity to:

- Document farmers current stewardship of the environment and their aspirations
- Identify realistic and achievable on farm environmental improvements
- Identify external water quality concerns and engage with landholders in the catchment
- Increase profits by identifying business development opportunities
- Increase exposure to grants and likelihood of successful applications

Since the launch of the project in April 2011, seven estuaries have committed to developing new EMS, whilst another two have requested reviews of their existing document. Upon completion this will take the total of estuary-wide Environmental Management Systems to sixteen, almost half of oyster producing estuaries in New South Wales.

Improvement of hatchery mollusc seed production: REPROSEED European Project

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Although academic knowledge is reported in scientific publications, practical progress in bivalve aquaculture has relied largely on empirical approaches. This situation is particularly acute for bivalve hatcheries-nurseries, as this activity has developed quite recently (□ 30 years) and the limiting factors have never been considered systematically. Such hurdles are often species-specific and concern different stages of the mollusc biological cycle. They mainly concern: Broodstock management and gamete quality, Reliability of larval rearing methods, Metamorphosis synchronization and improvement of settlement success, Quality of seed in terms of immunity, genetic diversity and sanitary status. Improvements of knowledge in these areas will undoubtedly lead to better hatchery methodology that will improve the reliability of spat production. The main objective of the REPROSEED project concerns the reliability and the capacity of hatcheries to respond to an increasing demand for mollusk seed, resulting from high variability of spatfall due to fluctuating environmental conditions in the wild. Moreover, using optimized hatchery-nursery rearing techniques, mollusk genetic improvement through selective breeding and/or polyploidization could be developed under controlled conditions. Finally, continuing interaction with the end-users in this project will help the transfer of knowledge and new technology and thus the development of more efficient European shellfish hatcheries.

Four bivalve species are targeted due to their economic importance in Europe and the different biological challenges they represent (*e.g.* different types of reproduction or settlement, differential sensitivity to bacteria at the larval stage, etc.), and an overall decrease in hatchery seed production cost is expected. This project centers its research on Pacific oyster and three ‘emerging’ species in hatchery production:

- The Pacific oyster *Crassostrea gigas*, for which scientific knowledge is the most advanced, resulting in the most important commercial seed supply activity in Europe.
- The king scallop, *Pecten maximus*, presently hatchery-produced in France and Norway, although not all of its rearing difficulties have been overcome. Due to its high susceptibility to bacteria, it is an excellent model for developing specific research in the field of prophylaxis (*i.e.* reducing the need for antibiotics).
- The blue mussels, *Mytilus edulis* and closely-related *M. galloprovincialis* are of major importance for the European shellfish industry, with an increasing demand for seed availability in the Netherlands, Spain and France, where hatcheries are expected to play a significant role in the near future.
- The European clam *Ruditapes decussatus*, for which only a very small amount of technological development has been made compared with the Manila clam *R. philippinarum*.

REPROSEED was launched in April 2010 with 12 participants including 9 research teams from 7 countries (France, The Netherlands, U.K., Norway, Spain, Portugal and Italy), 2 end-users (commercial hatcheries) and 1 participant in charge of the RTD/enduser interface. The scientific work plan is organized into 4 work-packages closely linked to the bivalve biological cycle (maturation – reproduction, larval stage, metamorphosis and postlarvae) and 2 cross cutting axes devoted to knowledge improvement in genomics and microbiology.

The first results from REPROSEED mainly concern larval and spat rearing in a Recycling Aquaculture System (RAS). Such systems have been shown to be suitable for oysters but are not yet completely satisfactory for scallop larvae. Interestingly, microbial studies revealed high vibrio levels in biofilms of scallop larvae RAS. The outdoor mass production of microalgae in paddle-wheel raceways has been tested using different media that could decrease production costs. Compared with Conway medium (control) diatoms grew correctly with a basic nutrient formulation, while continuous cultures could not be maintained very long (10 days) due to contamination. For gamete quality assessment, physical (gamete size, spermatozoa movement, etc.), biochemical (ATP, protein and lipid total content) and molecular (gene expression by real time PCR) parameters were measured to assess gamete quality. The combination of the two best parameters, protein content and motility, appeared to be relatively well correlated with egg quality ($r^2 = 0.420$), while insulin gene expression and hatching rate were significantly correlated. The acquisition of new genomic resources in scallop, mussel and clam are in progress. Eggs, larvae (at different development stages) and post-larval tissues have been collected to establish mRNA libraries to be sequenced using Roche 454 technology. Thereafter, oligo-microarrays will be developed and used to compare some extreme conditions at different bivalve life stages. Selected genes will also be used to focus on certain functions, such as immune response and studied by qPCR.

Advances in hatchery production of flat oysters *Ostrea angasi*

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Increased domestic demand coupled with potential export markets has renewed interest in farming of the native flat oyster, *Ostrea angasi*, in the southern states of Australia. However low numbers and unreliable wild catch of native flat oyster spat has meant the flat oyster industry has become reliant on hatchery produced spat. One important economic development has been the hatchery production of single seed or culchless oyster spat, larvae are induced to metamorphose without attaching to a substrate.

Improvements in hatchery production have been achieved by quantifying seasonal availability of larvae through the monitoring broodstock for 12 months along a latitudinal gradient, from Merimbula Lake, Bermagui River, Wagonga inlet Southern NSW to Gogleys Lagoon on mid north NSW coast. Spatial variation in the length of the spawning season was evident, with the northern most location having brooding oysters for the longest duration. Using magnesium chloride, facilitated easy removal of larvae without deleterious effects to the broodstock and larvae can be routinely collected from sites up to 12 h travelling time away and brought to the hatchery for culture and settlement.

The need to produce single seed spat from the hatchery prompted investigation of several catecholamines that may induce metamorphosis of *O. angasi* larvae and as a "tool" to examine the effects larval rearing conditions on larval development. . Two catecholamines were successful in producing culchless spat and treatment of competent *O. angasi* larvae with 10⁻³ M epinephrine bitartrate or 10⁻⁴ M epinephrine for 1h has been adopted for routine commercial production.

The observed variability in response to catecholamines prompted investigation of the effects of rearing conditions on larval competency and development. The influence of algal diet, temperature and salinity on survival, growth and development of larvae was investigated. A series of uni, binary and ternary algal diet combinations using eight algal species commonly used in hatcheries. Diets composed of Isochrysis sp. (T. Iso) and Tetraselmis chuii in combination with either *P. lutheri* or *N. oculata* promoted the greatest larval growth, survival and development. Optimal larval growth, survival, and development was observed at temperatures of 26-29°C and salinities of 30-35. Studies are now underway to further elucidate the mechanisms of catecholamine settlement induction.

Comparative accumulation and depuration of paralytic shellfish toxins in Pacific oysters *Crassostrea gigas* and Sydney rock oysters *Saccostrea glomerata*

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Paralytic shellfish toxins (PSTs) are a major group of marine biotoxins, that have potentially severe impacts on humans if they consume shellfish that have accumulated these toxins. Four species of marine dinoflagellates isolated in Australian waters have been found to produce PSTs. Two of these species have caused blooms in temperate regions of south-eastern Australia, particularly New South Wales and South Australia, where Sydney rock oysters (SRO) and both diploid (2N) and triploid (3N) Pacific oysters (PO) are cultivated.

Shellfish species show marked inter-species variation in their capacity to accumulate PSTs. Further, differences have been demonstrated in PST accumulation between diploid and triploid Pacific oysters

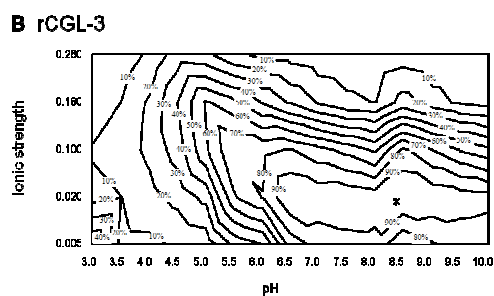
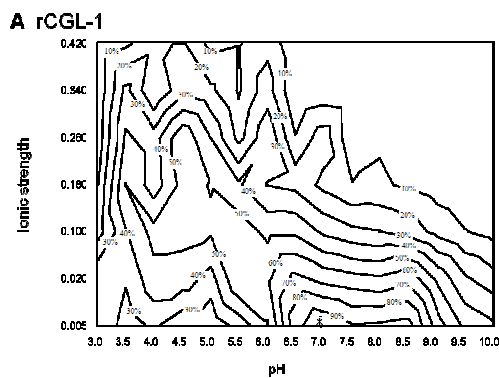
(Haberhorn et al., 2010). A series of trials are being undertaken to compare the accumulation and depuration of PSTs, between species (SRO vs PO) and types of oysters (2N vs 3N) at different temperatures. The outcomes of these experiments will be used to better inform both oyster farmers and regulators to permit the development of cost effective management protocols.

Multiple mantle lysozymes in the Pacific oyster serve important role for host-defense under broader environmental conditions

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Lysozymes are enzymes to cleave the glycosidic bonds in peptidoglycans forming Gram-positive bacterial cell walls. Recently, the presence of multiple lysozymes with various biochemical properties



has been demonstrated in several bivalve species. However, it is unclear whether these lysozymes could function as a defense molecule. Thus, in this study, to clarify lysozymes' function on host-defense mechanisms in the body of the Pacific oyster, *Crassostrea gigas*, we have characterized biological activity of lysozyme in mantle extracts, cloned and identified cDNA of two lysozymes (CGL-1 and -3), synthesized recombinant lysozymes (rCGL-1 and -3) with yeast *Pichia pastoris*. Furthermore, we also examined bacteriolytic and antimicrobial activities of these recombinant lysozymes. In the mantle extracts, greatly bacteriolytic activity of the mantle extracts against *Micrococcus luteus* was detected. Figures A and B show the effects of pH and ionic strength on *M. luteus* lytic activity of rCGL-1 and -3. rCGL-1 showed the highest lysozyme activity at pH 7.0 and an ionic strength of 0.005 in the measured range. rCGL-1 keeps relatively high activity within a broad range of acidic condition. In contrast, rCGL-3 expresses more than 70% of its maximum activity in the pH ranging from 6.5 to 10.0. rCGL-3 showed the highest lysozyme activity at pH 8.5 and an ionic strength of 0.020. Both rCGLs showed antibacterial activity against several species of Gram-positive bacteria but not inhibit the growth of the Gram-negative bacteria tested. rCGL-3 showed complete inhibition on the growth of two species of marine bacteria at 10 μ g/ml. rCGL-1 was less significantly effective than rCGL-3. From these results, we concluded that CGL-1 and -3 in the mantle brought a complementary relationship between them and the difference was suited for the host-defense of *C. gigas* under broader condition.

Experimental triploid oyster production by chemical induction”

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The report presents the results of triploid oyster production trials, in which two experiments on the effect of fertilizable temperature and chemical concentration to triploid rate, hatching rate, growth and survival of the larvae conducted in order to perfect the method to create triploid oyster.

Triploid rate, size of larvae collected at the highest concentration of 0.5ppm (41.34±6.35% triploid, the height reached 336.6 ±13.64 μm), following at concentration of 0.25 ppm (36±4.16% triploid, the height reached 326.67 ± 3.75 μm), at the lowest concentration of 0,1 ppm (25±2.89% triploid, the height reached 303.3 ± 3.33 μm). Hatch rate of chemical inductions are lower than that of control one. The survival rate of larvae is inversely proportional to chemical concentrations, lowest at concentration of 0.5 ppm(0.53 ±0.09), highest at the control (13.73±3.38).

Triploid rate, size of larvae when fertilization at temperature of 25°C is higher than that at 29°C. The survival rate in the fertilized plot at 29°C is higher than in plot fertilized at 25⁰C.

Oyster information portal – a novel tool for improved oyster industry management, governance and knowledge

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The Australian oyster industry relies heavily on the state of the environment, with a need for productive, healthy waters in the surrounding catchments and ocean. Increasingly this is becoming difficult as a result of coastal development, despite industry efforts to improve their practices and monitor the health of their waterways. Increased uncertainty related to climate change exacerbates industry issues related to the environment, and although oyster growers are aware of the relationship between the environment and productivity, the specific links and risk indicators still need to be better understood.

This project aims to take stock and consolidate environmental and industry knowledge that is otherwise diffuse, difficult to access and complex to interpret. Extensive environmental data and information resides across multiple sectors and jurisdictions around catchments and estuaries. In addition, the establishment of data collection systems for industry productivity, disease events and management practices has been undertaken. This approach brings together environmental and industry data in a format where, for the first time in NSW, farmers might be able to link environmental changes in and around their leases to productivity. An Oyster Information Portal (OIP) is being developed to deliver this consolidated information an industry identified priority, with the overarching aim to help the oyster industry develop strategies and practices to prepare for climate change and pinpoint some of the potential impacts on the industry.

A number of environmental factors have been identified (based on a review of the literature) that have the potential to affect oyster health. These factors include water temperature, salinity, pH, environmental flows, and chemical stressors among others. Changes in these factors result in a variety of responses within the oysters. These include oyster spawning events, propagate success, growth pulses, immune responses and susceptibility to disease, biochemical and metabolic reactions. By collating information on both the environmental factors and the oyster responses within the OIP, the oyster industry will be informed to make decisions and develop strategies that can be used to better plan for, and respond to, climate and catchment induced changes.

In our presentation we will demonstrate the OIP and explain the application of the tool from researcher, industry and stakeholder perspectives, to manage change and ensure the survival of one of Australia's most sustainable and high profile seafood industries.

Monitoring our oysters using automated oyster graders

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While large, well-shaped oysters have a high market value, they require optimal growing conditions and labour intensive techniques to culture. To increase handling efficiency many growers are investing in automated oyster graders that sort oysters photographically for pre-market. While graders are commonly used for sorting purposes they have the potential to be used to assist in monitoring the productivity of oyster cohorts and leases.

Pilot trials were undertaken which demonstrate the utility of oyster graders to answer some of the common production questions asked by growers in regards to cultivation methods, frequency of handling and stocking densities (Rubio, 2010 'Using an automated oyster grading machine for long-term monitoring of oyster performance'). However, no standardised industry-led national monitoring programs exist in Australia, and such a monitoring program is a pre-cursor to industry wide production and risk analysis, including responses to environmental shifts.

Our aim was to determine the feasibility of establishing a long-term monitoring program through a series of trials in four NSW estuaries that utilise automated graders. Information currently collected includes oyster growth and mortality of tracked oyster cohorts at different locations within each of the four oyster producing estuaries. The application is being trialled for both NSW farmed species *Saccostrea glomerata* and Triploid *Crassostrea gigas*.

Preliminary findings suggest that the return on investment of time into establishing these programs is quite small as it can be aligned within the day to day operations of farm management, and that the output of information provides for improved business planning and management responses. In most cases oyster growers have a good understanding of their estuaries, however by monitoring and quantifying oyster performance and mortality, growers will be able to characterize growing sites and identify unusual mortalities or lack of growth. Ultimately, long-term records can be related with climatological or environmental data in order to identify optimal conditions for high oyster production.

Oyster farming in Malaysia in relation to other Asean countries: challenges and successes

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Oysters have been harvested for food by the coastal communities for centuries throughout most ASEAN countries. However, little has been reported on the challenges and successes faced by growers. Oysters (*Crassostrea iredalei*, *Crassostrea belcheri* and *Saccostrea cucullate*) have been collected by local fishermen for several decades from intertidal rocks, estuarine river bottom, jetties and fishing stakes in coastal areas and islands throughout Malaysia. However, compared to other bivalve molluscs, oysters are relatively unknown in Malaysia due to its low production and lack of publicity, whereas oysters are commonly been consumed in Thailand. Under the auspices of the Bay of Bengal Programme (BOBP) (1988 – 1993) and International Development Research Centre, Canada (IDRC) (1989-1993), the Department of Fisheries Malaysia and several local institutions undertook the research on oysters and introduction of oysters farming in some selected areas in

Malaysia, as well as in Thailand, Indonesia and the Philippines. The programme eventually failed because the marketing aspect of oyster farming was not been considered in the programme and lack of natural oyster seeds to support the industry. Almost all the participants of the oyster programme stopped their oyster farming activities when the support from BOBP and IDRC had ended. The current oyster production in Malaysia is 2,128 tons in 2009 and this represents only 14% of the demand in the country. Most of the oyster seeds used for farming is harvested from the wild and currently Malaysia is facing serious problems in sustaining the industry due to insufficient seed stock. There was a stage that Malaysia was importing natural seeds from Thailand and Myanmar, and now facing difficulties in obtaining natural seeds from these countries. Oyster farming in Thailand is far ahead of Malaysia, Indonesia and Vietnam, where Thailand is able to export its oysters while the other ASEAN countries still rely on imported oysters. The expansion of oyster farming industry in Malaysia could much be faster if not because of limited seed supply. Only hatchery production can provide the required supply of seed both in term of quantity and quality, for the expansion of the farming industry. The research on oyster seed production had been initiated in Malaysia since 1989 under the programme by IDRC and the 5th and 6th Malaysian Plan. The first pilot hatchery for oyster seed production has been successfully set-up in 2009. This may be the only two commercial oyster hatcheries in ASEAN, with the other hatchery located in Vietnam. With the availability of hatchery-produced oyster seeds, oyster farming is blooming in the coastal areas of Malaysia. The details of the challenges and successes will be discussed.

Study of reproductive biology *Saccostrea cucullata*, in Serangan coastal, Bali Indonesia.

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Saccostrea cucullata is one of the tropical species of oyster are often found in coastal areas of Bali. Despite the high value commercially, but there is no information on the reproductive biology of this oyster. The purpose of study is to analyze the morphology and gonad maturity level *Saccostrea cucullata*. Gonad maturity level is divided into 4 stages from stage 1 to stage 4. The samples were taken every month for one year. The observation showed that the average length was 53.81 mm shells and shell width was 37.72 mm. the highest level of gonad maturity (stage 4) oyster was the case from August to October. The result showed that the oyster can reproduce throughout the year without being overly influenced by the changing seasons.

Digestive enzyme activities in the digestive diverticula of the Pacific oyster, *Crassostrea gigas*

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In *C. gigas* and other marine bivalves, the digestive diverticula consist of two types of ducts and



Fig 1 Enzymes detected in the digestive diverticula of *C.gigas*

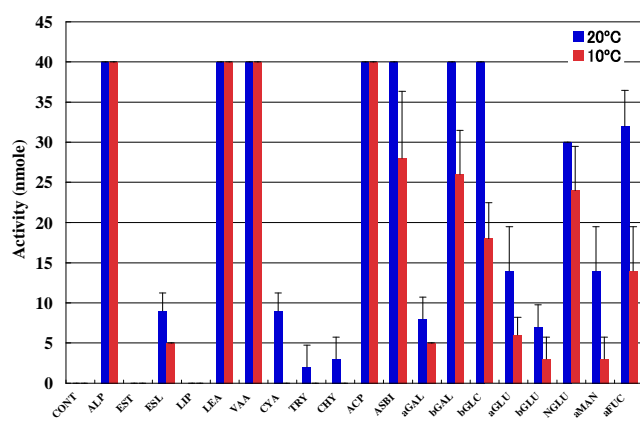


Fig 2 Seasonal difference in activity of enzymes within *C.gigas*

the supernatant by using the API ZYM system. Samples were incubated for 3 h at 10°C and 20°C. The enzyme activity was determined visually and ranked from '0' (0 nanomoles of hydrolysed substrate; no activity) to '5' (40 nanomoles of hydrolysed substrate; highest activity) according to the manufacturer's instructions. Using the API ZYM system, 17 enzymes of 19 enzymes assayed were detected in the digestive diverticula (Fig. 1).

Many enzymes showed significant seasonal differences in activity, only 12 enzymes were detected in winter. In contrast, leucine arylamidase displayed the highest activity (40 nanomoles of cleaved substrate) in both summer and winter. In difference in incubation temperatures, activities of samples incubated at 20°C were much higher than those of samples incubated at 10°C (Fig. 2).

Oyster Shelf-Life = Time and Temperature

Mark Tamplin* and Judith Fernandez-Piquer

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Vibrio parahaemolyticus is a natural bacterium that lives in marine environments. It is accumulated in oysters and may reach levels that cause human illness when post-harvest temperatures are not properly controlled and oysters are consumed raw or undercooked. Although the majority of *V. parahaemolyticus* strains do not present a risk to humans, some can cause mild to severe diarrhea. This typically occurs when levels of *V. parahaemolyticus* grow in oysters to high infectious levels.

In recent years, the number of *V. parahaemolyticus* outbreaks has increased. These events have been linked to changes in ocean currents and to elevated seawater temperature. In addition, most outbreaks have been caused by a *V. parahaemolyticus* strain that emerged in Asia in the 1990s. As a result, there

numerous blind-ending tubules (digestive tubules), the epithelia of which are composed of digestive cells and basophilic cells. These cells produce and secrete many digestive enzymes, therefore digestive tubules appear to be specialized for digestion of small food particles. To present and characterize, in relation to digestive function, enzymes in the digestive diverticula, we monthly measured enzymatic activities of cultured oysters through one year.

Specimens of *C. gigas* were obtained from a hanging-cultured bed in Miyagi Prefecture. Digestive diverticula were collected from shucked oysters. The collected samples were homogenized and centrifuged at $\times 9,000$ g for 10 min and the supernatant was submitted to enzyme assays. Activities of 19 hydrolytic enzymes were measured in

has been increased demand for practical risk management tools that can be used by oyster companies and health agencies to manage *V. parahaemolyticus* in oysters.

Temperature is an effective control that limits the growth of *V. parahaemolyticus* in oysters, both in growing waters and post-harvest. We can take advantage of this by designing temperature controls in supply chains that maintain *V. parahaemolyticus* at levels that meet market and safety standards.

Prior to this project, there was little information about how temperature affects the rate of *V. parahaemolyticus* growth in Australian Pacific and Sydney rock oysters, as well as the other natural bacteria in oysters that can limit shelf-life. The Australian Seafood CRC *Oyster Refrigeration Index* project addressed these needs and produced predictive models to help oyster companies forecast the effects of environmental conditions on the quality and safety of oysters, both on- and off-farm.

The model was produced by injecting Pacific oysters with a mixture of *V. parahaemolyticus* strains and then measuring growth rates from 4 to 30°C. Next, the data were translated into a mathematical model that was incorporated in a Microsoft Excel[®] workbook. In addition, a website was produced to facilitate access.

The model predictions were field-tested with Pacific oysters which contained natural populations of *V. parahaemolyticus*. Field tests demonstrated that the model provided fail-safe predictions for *V. parahaemolyticus* growth in Pacific oysters. Interestingly, *V. parahaemolyticus* did not grow in Sydney rock oysters between 4 to 25°C, which may lead to more flexible ways of safely handling this oyster species.

The *Oyster Refrigeration Index* will be useful to companies that have long supply chains, especially those shipping to international markets with maximum *V. parahaemolyticus* limits. Industry evaluation has shown that the model can be used to identify operations that have the greatest effect on product shelf-life and safety, design more efficient ways to cool oysters, and to demonstrate to employees how time and temperature are critical to oyster safety and quality.

Genetic diversity of Suminoe oyster in the Ariake Sea, Japan

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Although wild individuals of Suminoe oyster *Crassostrea ariakensis* have been a limitedly occurred and valuable fishery resource in Ariake Sea, Kyushu, Japan, for the last several decades, *C. ariakensis* has been recently designated an endangered species due to decrease in abundance and population. However, little is known about its biological and ecological properties in the native range, because even less research has been done on its reproductive and genetic structure in Ariake Sea. The aim of this study was to demonstrate annual changes in the genetic diversity of *C. ariakensis*. A total of 305 oyster specimens with the morphology of *C. ariakensis* were collected from the estuary of Kashima River along the northern coast of Ariake Sea in each September in 2006, 2008, 2009, and 2010. They were then subjected to species identification by multiplex-PCR analysis of the mitochondrial COI gene, and 299 specimens were identified to be *C. ariakensis*. From 34 specimens of the 2006 cohort, 38 ones of the 2008 cohort, 40 ones of the 2009 cohort, and 47 ones of the 2010 cohort, phylogenetic analysis based on nucleotide sequencing of the COI gene provided 22 haplotypes including 7 common haplotypes. The haplotype diversity (*h*) values were calculated to be 0.8235 ± 0.0401 and 0.7240 ± 0.0557 from the 2006 and 2008 cohorts, respectively, and thus *C. ariakensis* could maintain comparatively high level of the genetic diversity between 2006 and 2008. Meanwhile, the *h* values were calculated to be 0.2359 ± 0.0880 and 0.3802 ± 0.0897 from the 2009 and 2010 cohorts, respectively, indicating a significant reduction in its genetic diversity after 2009. In addition, the haplotype networks dramatically altered from the lineage of some common haplotypes in the 2006 and 2008 cohorts to the radiation from one major common haplotype HT01 in the 2009 and 2010

cohorts (Fig. 1). This rapid decline in the genetic diversity after 2009 allows the northern coastal population of *C. ariakensis* in Ariake Sea to occur genetic bottleneck. In order to form a framework for designing ecological management programs for valuable wild stocks of *C. ariakensis* in Ariake Sea, continuous genetic monitoring should be conducted.

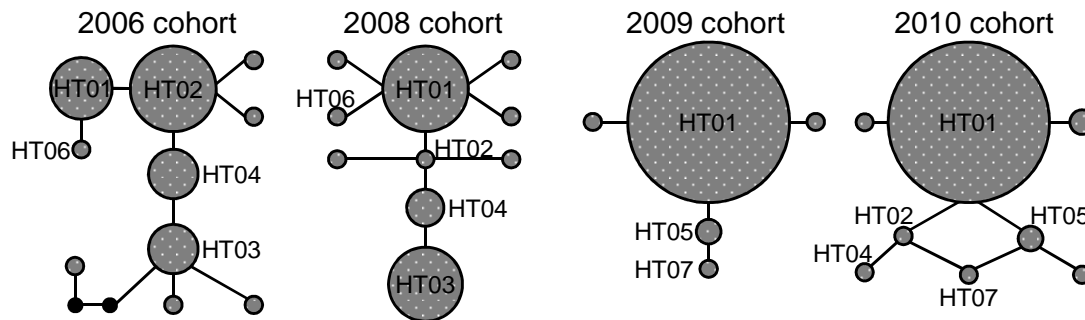


Figure 1. Haplotype networks of the 2006, 2008, 2009, and 2010 cohorts of *C. ariakensis* in Ariake Sea. Circle size is commensurate with the haplotype frequencies. A branch corresponds to a nucleotide substitution, and black circle

Effects of metal contamination on the expression of immune- and stress- response genes in Sydney rock oysters

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Environmental contamination by chemical pollutants is a serious threat to the biological sustainability of coastal ecosystems worldwide. Our current understanding of the biological effects of chemical pollution in these ecosystems is poor. Extensive chemical maps of pollution exist, but there is little corresponding biological effects data. New, more sensitive biomonitoring methods are needed to provide an early warning of biological harm that can assist in the management of sensitive marine environments and prevent permanent damage. The current study tests the expression of immune- and stress- response genes in Sydney rock oysters (*Saccostrea glomerata*) exposed to metals under controlled laboratory conditions.

Seven target genes (HSP70 & HSP90, metallothionein, superoxide dismutase, defensin, ficolin and ferritin) were tested. Quantitative (real time) PCR analyses showed that laboratory exposures to different metals (cadmium, copper, lead and zinc; 100µg/l) elicited different profiles of gene expression (Figure 1). Exposure to cadmium up-regulated the expression of HSP90 (a generic stress-response protein), but decreased the expression of defensin (an antimicrobial peptide), ferritin (a metal binding protein involved in stress responses), superoxide dismutase (SOD, an antioxidant enzyme involved in phagolysosomal defence) and metallothionein (another metal binding stress response protein). In contrast, copper exposure led to decreased expression of six genes (including ficolin, a lectin involved in host defense), excluding SOD. Lead down-regulated the expression of defensin and HSP70, whilst exposure to zinc decreased the expression of HSP70, metallothionein, defensin and ferritin, but upregulated HSP90.

	Cd	Cu	Pb	Zn
Defensin	↓	↓	↓	↓
Ferritin	↓	↓		↓
HSP70		↓	↓	↓
HSP90	↑	↓		↑
SOD	↓			
Ficolin		↓		
Metallothionein	↓	↓		↓

Figure 1. Patterns of gene expression in oysters exposed to 4 heavy metals (100µg/L, 4 days). Arrows indicate significant ($p < 0.05$) up- or down-regulation.

These results suggest that metal exposure has complex, differential effects on the immune- and stress-responses of oysters that could provide a mechanistic understanding of the effects of specific stressors. The significance of these data is now being assessed using oysters exposed to contamination in the field.

Influence of serotonin and norepinephrine on induction of larvae settlement of tropical oyster larvae, *Crassostrea iredalei*

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Serotonin (5-HT) and norepinephrine (NE) are two neurotransmitter compounds that can be found in most invertebrates either during larvae stage or adult in low levels. Larval settlement of tropical oyster, *Crassostrea iredalei* was investigated by exposing the eye-spot larvae to five different concentrations (10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} M) of 5-HT and NE for 1 and 24 hours. Control group of the study was the larvae exposed to 1µm filtered seawater. From the results, more than 50% to 80% of the larvae settled and cemented on the substrate when exposed to 10^{-5} M NE for 1 hour. About 55% cemented spat was recorded when exposed to 10^{-6} M NE for 24 hours. 30% to 60% of the cemented spat were recorded from the larvae exposed to 10^{-6} M 5-HT. In this study, NE had effectively induced the cemented spat compared to 5-HT. Results showed significant differences between the control group and treatment groups. This study would provide useful information technique of seed production in hatchery.

Effects of metal contamination on Sydney rock oysters: a proteomic approach

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Chemical pollution has significant biological impacts on industrial and urbanised coastal ecosystems. It is imperative to develop effective methods to monitor these impacts on native biota and their environments. Among a suite of biomonitoring techniques, molecular biomarkers have the potential to link contaminants directly to their effects on biota. However, traditional molecular biomarker analyses can be insensitive, especially at low contaminant levels. Proteomics may provide a method

for identifying the biological effects of pollution at extremely low levels of contamination over short time periods.

The current study uses proteomics to assess the effects of metal contamination on Sydney rock oysters. Oysters were exposed to three environmentally relevant concentrations of cadmium, copper, lead and zinc (100 µg/l, 50 µg/l and 5 µg/l) over four days under controlled laboratory conditions. Oyster hemolymph from metal-exposed oysters was then compared to hemolymph from non-exposed controls by 2-dimensional electrophoresis to identify differentially expressed proteins. Differential proteins were characterised by tandem mass spectrometry (LC-MS/MS) so that their putative biological functions could be assigned.

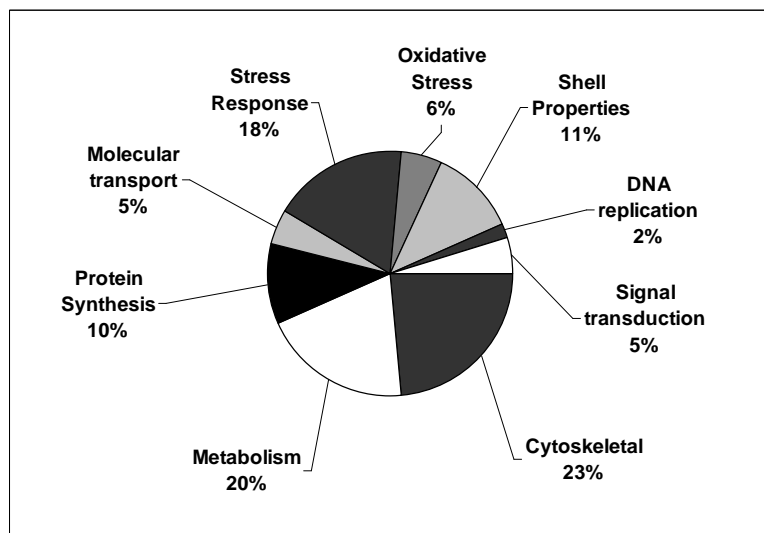


Figure 1 – Putative biological functions assigned to proteins that differed in expression ($p < 0.05$ relative to controls) in response to 5, 50 and 100 µg/l of cadmium, copper, lead or zinc

The concentrations of 129 proteins were significantly altered by metal exposure. The data suggest that there are unique protein signatures associated with exposure to each metal and each concentration of metal, even though there was some overlap between treatments. Differential proteins were putatively assigned to 9 different functional categories, of which cytoskeletal activity accounted for 23% (Figure 1). Ongoing work includes testing the efficacy of these potential protein biomarkers in the natural environment.

Mudworms in Australia – what’s in a name?

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Mudworm disease in the Australian oyster industry was first identified in 1885 after dramatic stock losses in estuaries along the east coast. One hundred and thirty years later the exact identity of these disease-causing pests remains unclear. This gap in basic taxonomic knowledge limits further research and development of solutions to the mudworm problem which could greatly assist in the development of a healthy and sustainable oyster industry.

This presentation introduces the mudworms (Polydora-complex species) associated with oysters in Australia and the problems associated with making reliable species identifications. Options to address these taxonomic problems are suggested. The current state of knowledge of mudworm research including morphological and molecular identification, mudworm host species distribution and country of origin of introduced species is presented, including the results of taxonomic research on mudworms from previously un-sampled oyster producing estuaries.

Being able to assess the mudworm disease problem from a solid taxonomic basis allows us to address questions such as: Which mudworm species are introduced and which are native? How can we control further introductions? Have the native species become pests through the actions of humans? What are possible control or remediation measures? Are particular mudworm species associated with other oyster diseases? What are the ecological and reproductive requirements of each pest species and can we use this knowledge to re-establish sub-tidal farming techniques and increase oyster production area?

Oysters in a changing climate: strengthening resilience through effective governance responses

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Governance structures and organisational arrangements for the oyster industry in Australia are complex with multiple government authorities and industry bodies involved. There are overarching legislation and policy statements at the national level, however, the industry is primarily regulated at the state and local government levels. Focus areas for regulation are planning, compliance and environmental health. Although a relatively integrated approach to governance and regulation has been adopted through the Oyster Industry Sustainable Aquaculture Strategy (OISAS) in NSW the impacts of climate change on the local oyster industry will present further governance challenges. Key issues addressed in this presentation will be the extent to which the regulatory frameworks and governance structure for the industry both nationally and in NSW have incorporated climate change impact and sensitivity issues and how these structures and arrangements are responding to them. In examining these questions it is particularly important to identify channels of information on climate change impact and sensitivities for oyster farmers, ways in which they can represent their concerns in relation to climate change impacts and sensitivities and options for regulatory and governance responses to these climate change impacts and sensitivities.

Three categories of stressors that affect the NSW oyster now, and that the industry faces in light of climate change, have been identified. These stressors include increasing frequency or intensity of disease, interaction of climate change stressors with impact events, and the cumulative and interactive stressors of climate change and catchment stressors. Historical responses to similar challenges will be reviewed and scenarios considered as to how governance response systems can be applied and effective for future challenges associated with climate change. The implementation of an information tool, the Oyster Information Portal, and its potential application as a trigger for a chain of effective

governance responses will be evaluated through scenarios that include a disease event, an impact and a cumulative stress of catchment change.

The questions addressed in the presentation will include:

What information (e.g. evidence of water quality impact, evidence of productivity declines) does the industry require to respond to events through management and communication with governance?

What information do governance agencies require to respond to an event?

What sort of long term base-line data is important for the industry to access to demonstrate long-term changes that require a governance response?

What are the long term governance strategies that could help the industry adapt to climate change?

Identification of a new anti-oxidant substance from Pacific oysters (*Crassostrea gigas*) and analysis of anti-oxidant capacity

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Radical oxygens oxidize constituent elements of biological body and damage cells. That are thought to be a possible causes of various disease such as cancer, arteriosclerotic disease, sugar diabetes, Alzheimer's disease, Parkinson's disease. Recent remarkable advance in anti-oxidant food is done to contribute prevention of these diseases.

We identified anti-oxidant substance from the extract of the soft-body sites of pacific oyster (*Crassostrea gigas*), and we have named that E6. E6 was isolated by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC), and was identified by UV absorption spectrometry, Nuclear Magnetic Resonance (NMR), Mass spectrometry (MS). As a result, we identified E6 as 3,5 – dihydroxy-4-methoxybenzyl alcohol. Furthermore, We measured ORAC value of E6 that is 1.24 ± 0.35 ($\mu\text{mol TE} / \mu\text{mol}$). ORAC value of E6 was higher than a-tocopherol (Vitamin E) and L + ascorbic acid (Vitamin C). E6 was estimated amphiphilic anti-oxidant from the structure.

We reported to Oyster extract cause a dose-dependent reduction of 8-OHdG that is oxidative product of guanine base in the past. E6 may contribute to one factor of this phenomenon.

Space Invaders: *Crassostrea gigas* not presently replacing native oysters along QX disease - infected rocky shores

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In Australia, the Sydney rock oyster *Saccostrea glomerata* suffers mass mortality from QX oyster disease, caused by haplosporidian parasite, *Marteilia sydneyi*. The non-native oyster *Crassostrea gigas* is QX-disease resistant, is already found in some New South Wales estuaries and has been suggested as a commercial cultivation option. However, *C. gigas* is the most invasive oyster species worldwide, sometimes displacing native species and destroying habitats. Therefore, knowledge of the ecological risks of *C. gigas* cultivation in Australia is essential for effective industry and ecosystem management. Where QX disease increases mortality among wild *S. glomerata*, invasion of *C. gigas*

might increase, potentially causing dramatic ecological impacts. We tested this hypothesis in areas immediately adjacent to those where QX disease has caused up to 90% mortality among cultured oysters since 2004 and in which wild populations of *C. gigas* exist. We found that despite impacts of QX on cultured oysters, apparent rates of mortality of wild *S. glomerata* were much lower, and abundances of *C. gigas* were generally low. Our results indicate that QX disease is not presently reducing biotic resistance of east Australian systems to *C. gigas* invasion. However, the spread of existing or new aquatic diseases might affect the longer term biotic resistance to invasive marine species.

Predicting the physiological response of oysters to climate change

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The earth's oceans are acidifying and warming with potentially devastating consequences to marine organisms and their ecosystems. Marine and estuarine molluscs have been found to be particularly susceptible with studies finding a range of negative effects across all life-history stages; including reduced calcification, growth and survival of adults and increased abnormality and development time of larvae. These effects are exacerbated when the two factors, elevated temperature and carbon dioxide, act synergistically. There remains a paucity of information documenting the complex nature of the impact of climate change on marine molluscs. This is largely because studies have found species-specific differences in responses, even between closely related species. Also the physiological mechanisms that are associated with the differences in marine molluscs' responses to ocean acidification are virtually unknown. Among Ostreids, Parker et al., (2010) found that the effects of elevated CO₂ and temperature were greater for Sydney rock oysters, *Saccostrea glomerata*, than for Pacific oysters, *Crassostrea gigas*. Most recently it has been suggested that oysters with a greater metabolic rate and feeding efficiency may be resilient to the impacts of climate change (Parker et al., 2011). It is known that the metabolic efficiency and feeding rate of adult *C. gigas* is greater than *S. glomerata* under ambient conditions (Bayne et al., 1999), but it is unknown how metabolic rate and feeding efficiency will be altered under elevated CO₂ and temperature. Deciphering the underlying physiological mechanisms through which mollusc species respond to climate change stress is, therefore, of great interest. This study aims to determine and compare the effects of ocean acidification and warming on the feeding efficiency and metabolic rate between two ecological and economically important oyster species in Australia, *S. glomerata* and *C. gigas*. Adults of the two species were reared at two carbon dioxide (CO₂) levels (pCO₂, 385 µatm {ambient} and 1000 µatm {elevated}) and two temperatures (22 °C {ambient} and 28 °C {elevated}), selected based on projections by the Intergovernmental Panel on Climate Change for ambient atmospheric pCO₂ and temperature levels for the year 2100. The synergistic effects of ocean acidification and ocean warming on a range of physiological parameters (clearance rate and rate of ingestion, absorption efficiency and absorption rate, oxygen consumption, excretion rate, oxygen consumption rate, oxygen: nitrogen ratio, mass growth, extracellular pH, condition index and scope for growth) of the two species were measured. Once concluded, this study hopes to provide a greater mechanistic understanding of the reasons underlying the response of the Pacific oyster compared to the Sydney rock oyster and how climate stressors impact on valuable marine and estuarine organisms important for our aquaculture industry.

Oyster Culture development in Northern Vietnam

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Vietnam has some 3,260 km of coastline harbouring many native clams, mussels and oysters with excellent production potential. In general, mollusc culture efforts have focused mainly on clams with approximately 190,000 t produced per annum. Until recently oyster production was small and almost entirely from the wild fishery. For both clams and oysters, any expansion of production was limited by seed availability.

To meet seed demand, scientific and commercial interest in Vietnam turned to hatcheries and to promote development, the Research Institute for Aquaculture No1 (RIA No1) constructed a mollusc hatchery at the National Marine Broodstock Centre (NMBC), Cat Ba, Northern Vietnam. Through collaborative research funded by the Australian Centre for International Agricultural Research, researchers at RIA No1 have adapted existing production technologies and established a rapidly growing oyster industry.

From humble beginnings of approximately 20 million seed in 2007, the NMBC now produces in excess of 100 million seed per year and have assisted in the development of 3 additional commercial facilities. Concomitant with increased seed supply, oyster production has also increased rapidly. In the first year approximately 100 t of oyster were produced from NMBC seed. This grew to 1000 t in 2008/09, which doubled to 2000 t in 2009/10. The current production estimate for 2010/11 is 5000 t with continued expansion in future years.

Researchers at RIA No1 are now turning their attention to increasing single seed production; improving oyster quality; increasing oyster health diagnostic capacity; increasing phytosanitary assessment capacity and evaluating new nursery and growout techniques.

Population genetics of *Crassostrea hongkongensis* along the coast of South China Sea inferred from mitochondrial genes and microsatellite loci

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The Hong Kong oyster, *Crassostrea hongkongensis* is a primary cultivation species and fisheries resource in the coast waters of South China Sea. Despite the significant advances made on biological and taxonomic aspects of this species, no detailed studies of population genetics have been carried out to understand its genetic diversity and genetic structure over the distribution areas stretching roughly 1,600 kilometers long along the coastal line of South China Sea. In this study, analyses of mitochondrial gene sequences and microsatellite loci were used to investigate genetic variations and structure of *C. hongkongensis* native populations collected from eleven locations along the coast of South China Sea.

A total of 62 haplotypes in *cox1* and 57 in *nad5*, respectively, from 230 individuals were identified through direct sequencing, with some associated with geographical regions; while 205 alleles were observed in 12 microsatellite loci from 627 individuals using capillary electrophoresis genotyping with ABI 3130 genetic analyzer. The pairwise *F_{ST}* from microsatellite markers ranges from 0.034 to 0.124, and those from sequence data range from -0.013 to 0.413 for *cox1* and -0.001 to 0.135 for *nad5*, respectively. Significant global Φ_{ST} from microsatellites and from sequence data indicate genetic heterogeneity among populations. Both AMOVA and *F_{ST}* analysis from microsatellite and sequence data revealed significant population structure, though microsatellite markers display a reduced level of differentiation among samples. The highest local differentiation was observed between the sample pools from Guangxi versus Guangdong and Fujian, which are separated by Leizhou peninsula.

Based on Structure analysis, NJ tree reconstructed from the microsatellite data and parsimony networks analysis of the two mitochondrial genes, it is concluded that *C. hongkongensis* native populations consists of a set of geographical clades from Guangxi (include Zhanjiang from Guangdong), Guangdong and Fujian. Pairwise $F_{ST} / (1-F_{ST})$ plotted against pairwise geographic distances among 11 populations shows a positive correlation, suggesting a genetic pattern of isolation by distance (IBD). The Mantel test confirmed that the association between genetic distance and geographic distance (km) was statistically significant. The findings from the present study could be useful for the genetic management of *C. hongkongensis* populations and may serve as a baseline in monitoring future genetic changes in their genetic diversity, either due to natural or anthropogenic impact.

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