Breeding Focus 2018 - Reducing Heat Stress

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Preface

“Breeding Focus 2018 – Reducing Heat Stress” is the third workshop in the series. The Breeding Focus series was developed to provide an opportunity for exchange between industry and research across a number of agricultural industry sectors. With this goal in mind, workshops have included presentations across the livestock and aquaculture industries to take participants outside their area of expertise and encouraged them to think outside the box. This year we increased the scope even further by also inviting presentations from the cropping and horticulture industries. Since the topic of heat stress has recently gained increased attention, we will discuss a wide range of aspects associated with heat stress, such as the physiology of heat stress and phenotypic indicators, genetic approaches and industry impacts.

Heat stress in animals describes a situation where an animal is exposed to high temperatures and unable to dissipate body heat, which causes an increase in body temperature. In the short term, an animal will react to heat stress with behavioural strategies (e.g. seeking shade, panting) to reduce the heat load. With prolonged excessive heat load, feed intake is reduced and production losses occur. Under extreme circumstances, excessive heat load can lead to death. In plants, heat stress can be defined as irreversible damage to plant function and development as a consequence of hot temperatures. Environmental causes of heat stress in plants and animals include high temperatures and high humidity over a long period of time, which is exacerbated by low cloud cover and high solar radiation.

With raising average temperatures, agricultural industries are faced with the challenge to manage potential impacts of heat stress on their crops, their pasture base and welfare and production of their livestock or aquaculture species. Management strategies such as shade and irrigation are effective but costly and, depending on the severity of climatic conditions, may have limited success. Susceptibility of organisms to heat stress can vary due to factors such as age and general health, but also genetic factors, such as breed or variety. Further, as we will hear during the workshop, genetic variation exists within breeds that enables genetic approaches to address heat stress in plants and animals. Selective breeding provides a long term approach that facilitates improvement of the physiology of plants and animals to cope with excessive heat load. The challenge here is to obtain cost-effective phenotypes to describe heat stress.

The chapters of this book discuss where the current climate is trending, and outlines opportunities for the crop, orchard, livestock and aquaculture industries to describe and measure heat stress, all with the focus on genetic improvement.

We would like to thank everyone who has contributed to this event for their time and effort: the authors for their contributions to the book and presentations, the reviewers who all readily agreed to critique the manuscripts. We would like to express a special thanks to Kathy Dobos for her contributions into the organisation of this workshop and the publication. Thank you!

Susanne Hermesch and Sonja Dominik
Armidale, September 2018

Summer mortality in molluscs: the genetic basis for resilience and susceptibility

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Abstract

Summer mortality is a phenomenon that causes mass die offs of molluscs during the summer months. Summer mortality affected molluscs include many economical important animals including; oysters, mussels, clams, scallops and abalone. The condition occurs in both natural and aquaculture environments and represents a considerable threat towards aquaculture and fisheries industries world-wide. Although sudden increases in water temperature are known contributors to summer mortality events, intrinsic factors (i.e. animal age and gametogenesis) and extrinsic factors (i.e. food availability and quality, increased salinity, decreased dissolved oxygen and the presence of pathogenic viruses and bacteria) can also play a role. Genetically driven factors are also considered to play a crucial role in susceptibility and resilience to summer mortality and these have been investigated recently using transcriptomics (i.e. broad scale patterns of gene expression). Immune, metabolic and stress response processes have commonly been found to differ between resilient and susceptible families, lines and/or populations. In addition, more complex genetic interactions such as regulatory transcription and epigenetic

processes may also influence summer mortality. Determining the functional mechanisms underpinning summer mortality resilience and susceptibility will enable that knowledge to be incorporated into selective breeding/genetic improvement programs.

What is summer mortality?

'Summer mortality' is a term used to describe the sudden, and often unprecedented, mass mortality of molluscs during the summer season. Molluscs affected include; oysters (Goulletquer *et al.* 1998; Samain and McCombie 2008), mussels (Goulletquer *et al.* 1998), scallops (Zhang and Yang 1999; Xiao *et al.* 2005), clams (Fiori *et al.* 2004) and abalone (Vandepeer 2006). A sudden increase in water temperatures is considered the most important factor responsible for summer mortality, however temperature alone is insufficient to explain the severity of mortality events. Complex multifactorial involvement of several intrinsic and extrinsic factors are known to contribute to summer mortality (Table 1). Elevated temperature appears to be the most important extrinsic factor. Food availability and quality (Tremblay *et al.* 1998; Delaporte *et al.* 2006; Pernet *et al.* 2010; Stone *et al.* 2014), increased salinity, decreases in dissolved oxygen and the resulting anoxic conditions (Oliveira *et al.* 2015) are generally considered to be contributing factors (Munari 2011). The prevalence of pathogenic bacteria and virus' during these heat stress anomalies has also emerged as a plausible key component of summer mortality syndrome, however the exact contribution these pathogens are having on individual reactions during a summer mortality event is unclear (Lacoste *et al.* 2001; Travers *et al.* 2008; Beaz-Hidalgo *et al.* 2010; Liu *et al.* 2017). Pathogens such as the ostreid herpes virus, are suspected to take advantage of immunocompromised individuals under certain stressful environmental conditions (Lynch *et al.* 2012). Complexities such as how the environmental factors impact the host, the pathogen and the host/pathogen relationship are also considered to have an effect on the severity of summer mortality (Solomieu *et al.* 2015). For example, King *et al.* (2018) found variability in the Pacific oyster, *Crassostrea gigas*, microbiome and oyster site location were significant factors in variability between summer mortality disease states of oysters. Changes in the relative abundance of bacterial groups including *Brachyspiraceae*, *Mycoplasma*, *Mollicute*, *Bacteroidales* and *Paludibacter* and *Vibrio* species were found to be significant factors in describing diseased oysters (King *et al.* 2018). Furthermore, bivalve molluscs (oysters etc.) are distant relatives of gastropod molluscs (abalone) are unlikely to be affected by the same pathogens in the same manner (Hooper *et al.* 2007).

Life stage has been demonstrated to play a pivotal role in the degree of summer mortality susceptibility (Lacoste *et al.* 2001; Samain and McCombie 2008; Stone *et al.* 2014). Summer mortality susceptibility has also been associated with the stress involved in reproduction which is generally considered to be related to the energetic burden that occurs during spawning and post-spawning events (Myrand *et al.* 2000; Li *et al.* 2007a; Huvet *et al.* 2010; Wendling and Wegner 2013). The animals condition can then be exacerbated by external factors such as pathogens (Wendling and Wegner 2013) and diet deficiencies (Tremblay *et al.* 1998). In *C. gigas* there is a change in the metabolic processes from carbohydrate storage to carbohydrate metabolism, resulting in depleted glycogen content and glycogen metabolism during spawning (Berthelin *et al.* 2000). After experiencing a heat shock event, *C. gigas* that have spawned also experience reduced synthesis of heat shock proteins (HSPs), reduced levels of glycogen stores in the mantle tissue and reduced adenylate energy charge, suggesting that there is lower energy available for metabolic activity (Li *et al.* 2007a). The cumulative effect of spawning and heat shock results in reduced haemocyte phagocytosis and hemolymph antimicrobial activity which may make individuals more susceptible to stressors such as infection or disease (Li *et al.* 2007a). Phagocytosis may also be impaired after spawning in *C. gigas*, thus post-spawning molluscs may be more susceptible to the effects of heat and bacterial stress (Wendling and Wegner 2013). Correlations have also been found between disease susceptibility and the maturation and spawning processes of *H. tuberculata*, with increased bacterial infection demonstrated after spawning (Travers *et al.* 2008). The sensitivity of adult and near adult molluscs poses a considerable risk to wild mollusc populations. The susceptibility of adult molluscs also poses a threat to the aquaculture industry, may experience economic loss due to the time and resources required to raise these animals to adulthood, only to have them die before harvest or breeding.

Table 1. Known variables associated with temperature rises and summer mortality

Classification	Species	Contributing Extrinsic factor	Contributing Intrinsic factors	Reference
Gastropoda, Haliotoidea, Haliotidae				
	Haliotis laevigata	Diet	Age	Stone et al. (2014)
	Halitois turbulata	Pathogens	Spawning	Travers et al. (2008)
	Haliotis laevigata	Diet		Duong et al. (2016)
Bivalvia, Mytiloida, Mytilidae				
	Mytilus edulis	Diet	Spawning	Tremblay et al. (1998)
	Mytilus galloprovincialis	Water chemistry	Pathogens	Romero et al. (2014)
Bivalvia, Ostreoida, Ostreidae				
	Crassostrea gigas	Pathogens	Spawning	Wendling & Wegner (2013)
	Crassostrea gigas	Pathogens	Age	Lacoste et al. (2001)
	Crassostrea gigas	Diet	Gametogenesis	Delaporte et al. (2006)
	Crassostrea gigas	Pathogens		Liu et al. (2017)
	Crassostrea gigas		Spawning	Huvet et al. (2010)
	Crassostrea gigas		Spawning	(Li et al. 2007b)
Bivalvia, Veneroida, Cyrenoidea				
	Corbicula fluminea	Water chemistry		Oliveira et al. (2015)

The research into summer mortality resilience is extensive, and so the research mentioned in this chapter focuses primarily on highlighting research specifically associated with identifying molecular genetic differences between susceptible and resilient individuals in an attempt to identify possible selective measures to combat summer mortality.

Economic significance of summer mortality

Molluscs are a vital food resource world-wide and are of high economic importance. In recent years a world-wide shift towards aquaculture and away from the fishing has occurred. A total of 104 mollusc species have been registered for farmed production (F.A.O. 2016). Mollusc aquaculture production world-wide in 2015 amounted to 16.4 million tonnes (0.3 inland and 16.1 marine/coastal aquaculture) at a value of US$18 billion (F.A.O. 2016). Aquaculture production of molluscs in Australia in 2015 amounted to 17,216 tonnes valued at US$100 million, with the three industries (edible oysters, Pearl oysters and abalone) accounting for 94% of total value (Savage 2015). Although small in comparison to agriculture, the value of these cultured species is extremely important to regional economies. In 1998, China experienced a 37% drop in production of the Zhikong scallop (*Chlamys farreri*) due to summer mortality, with an estimated loss of US$360 million (Gosling 2015). In 2003, Taiwan estimated the abalone (*Haliotis diversicolor supertexta*) stock loss due summer mortality at US$11.5 million (Chang *et al.* 2005). Summer mortality stands to threaten this vital economic resource. Summer mortality often impacts breeding adults and is costly to the aquaculture industry, requiring time and resources invested into culturing animals that die before they reach maturity. As well as the loss of stock, incidences of summer mortality may be highly costly to the aquaculture industry due to the decontamination of equipment and facilities that may harbor disease.

Over the past few decades, significant regional variation in the frequency of extreme oceanic thermal stress anomalies has been recorded (Selig *et al.* 2010). A record heat wave was experienced in the European summer of 2003, with surface water temperatures at least 2-3°C higher than previous summers in the uppermost 15 m of ocean throughout the coastal and central zones (Sparnocchia *et al.* 2006; Sorgente *et al.* 2007). This heat wave had a devastating effect on the benthic macroinvertebrate species in the Mediterranean, particularly along the coast of Italy (Garrabou *et al.* 2009) with molluscs representing one of the most affected taxa (Munari 2011). In 2011, the Western Australia coast experienced a sudden approximately 3°C increase above the long-term monthly average. This event was unprecedented and superimposed on a long-term temperature increase experienced in the region (Pearce and Feng 2013). The warming event peaked in March, with recorded water temperature increases of up to 5°C on 10m deep rocky reefs (Wernberg *et al.* 2013). Some populations of Roe's abalone (*Haliotis roei*), one of the most affected species, were recorded to be completely annihilated (Pearce and Feng 2011). Temperature associated mortality events are also not limited to the summer season. In the winter of 2014, high temperatures and turbidity, and low salinity, have been implicated to cause mass mortality event of blue mussels (*Mytilius edulis*) in a coastal inlet off the French Atlantic coast (Polsenaere *et al.* 2017). Nine thousand metric tonnes of blue mussels were lost with mortality rates of up to 100% recorded on mussel farms (Polsenaere *et al.* 2017). With global climate change heralding shifts in abiotic variables such as temperature, stress and mortality is likely to occur on a larger scale and frequency, seriously impacting mollusc aquaculture in the future (Helmuth *et al.* 2002).

Summer mortality preventative measures

Early attempts to manage summer mortality involved methods such as overstocking (to account for lost stock), transplanting animals out of areas before mortalities occurred, harvesting early (Hershberger *et al.* 1984) and reduced feeding to maintain water quality (Vandepeer 2006). However, these approaches can be counterproductive and ultimately resulted in loss of revenue and more advanced methods of prevention are needed.

Chemical management of water quality may assist in the management of mortalities. Limited oxygen availability is known to severely affect mollusc respiration activity at high temperatures, particularly in summer when water temperatures are higher and dissolved oxygen levels are lower. Oxygen concentrations need to be maintained at high levels to avoid hypoxic responses in molluscs, which can cause a reduction in oxygen consumption and feeding activity, and ultimately result in metabolic depression (Dunphy *et al.* 2006; Le Moullac *et al.* 2007). This is of particular importance to land based mollusc aquaculture industries where tanks and shallow flow through systems are at risk of low oxygen concentrations during the summer months.

Research into acclimatization of animals to more extreme temperatures suggests this can contribute to combating summer mortality (Li *et al.* 2012; Falfushynska *et al.* 2016). Pre-shock treatments, where animals are exposed to mild abiotic stress exposure prior to a major stress event may also represent a promising method to lessen the impact of summer mortality (Li *et al.* 2007b). These pre-shock treatments have the potential to invoke physiological acclimation and protect against future abiotic stressors and pathogen infection by “priming” or increasing the activity of stress response mechanism’s, such as the production of HSPs (Sung *et al.* 2008). Sub-lethal heat shock of the bay scallop, *Argopectan irradians irradians*, from 20°C to 32°C has been demonstrated to protect the animals against lethal treatment exposure at 35°C for 7 days after exposure (Brun *et al.* 2009). Feeding *Artemia* larvae with bacterial HSPs has been found to provide protection against pathenogenic infection (Sung *et al.* 2009). Heat shock protein inducing compounds such as those isolated from the prickly pear (*Opuntia ficus indica*) have also been found to protect *Artemia* against a range of abiotic stressors (Baruah *et al.* 2012).

Diet quality has been shown to be an active participant in preventing mass mortalities of molluscs. In filter feeding molluscs, i.e. *C. gigas*, beneficial algae such as diatoms have been correlated with energetic reserves and a decrease in disease mortality (Pernet *et al.* 2014). In grazing molluscs, such as abalone, macroalgae diets have been demonstrated to provide higher antioxidant capabilities compared to those fed a commercial diet (Wan *et al.* 2004). Such diets are highly effective at preventing mass mortalities of grazing molluscs, such as abalone, during summer mortality events (Stone *et al.* 2014; Bansemer *et al.* 2016) Additionally, grape seed extract has well documented antioxidant, antimicrobial and anti-inflammatory properties when ingested (Perumalla and Hettiarachchy 2011). In abalone, grape seed extract has been shown to act as an efficient dietary additive to improve productivity and reduce mortality during heat stress (Lange *et al.* 2014; Duong *et al.* 2016).

Genetically driven factors are also considered to play a crucial role in summer mortality susceptibility (Solomieu *et al.* 2015). The Pacific oyster (*Crassostrea gigas*) has become a model organism for summer mortality research, especially when comparing summer mortality resilient or susceptible families and populations, to identify the functional mechanisms that may be responsible for the variation in survival (Huvet *et al.* 2004; Samain *et al.* 2007; Lang *et al.* 2009; Fleury *et al.* 2010; Fleury and Huvet 2012; Rosa *et al.* 2012; Schmitt *et al.* 2013; Segarra *et al.* 2014). As well as known immune, metabolic and stress response processes (Table 2), more complex genetic interactions such as regulatory transcription (Heare 2015) and epigenetic processes (Fréchette *et al.* 2003) may be a factor in defining summer mortality resilience and susceptibility. The long-term adaptation of summer mortality affected family lines/populations may also be associated with past environmental conditions through epigenetic effects, with environmental factors having the potential to alter heritable changes such as DNA methylation, histone and chromatin process without changing the corresponding DNA sequence (Allis and Jenuwein 2016).

Selective breeding to combat summer mortality related stressors has had some success in increasing survival rates and has the possibility to improve resistance to summer mortality related factors (Huvet *et al.* 2004; Samain *et al.* 2007; Sauvage *et al.* 2009; Fleury *et al.* 2010; Fleury and Huvet 2012; Schmitt *et al.* 2013). However, summer mortalities are caused by a variety of different factors (Samain and McCombie 2008), and the contribution of each to the summer mortality occurring on farm might vary from event to event. Challenge tests manipulating water temperature and oxygen will have little effect on improving the resilience to summer mortality if increased pathogen load and pathogen susceptibility under high temperatures are the major cause of summer mortality, and if these pathogens are not present during the challenge test. It is difficult to improve a population's overall robustness when the factors contributing to robustness (eg. tolerance to high temperatures, low oxygen and overall disease resistance) are multifactorial and uncorrelated (Robinson *et al.* 2017). Breeding from survivors to natural or induced summer mortality events on-farm could be the best option for genetic improvement. Maintaining genetic diversity within breeding stocks will be an important consideration if multiple factors are involved.

RNA-seq and differential expression

Much of the research into summer mortality genetics has been described through gene expression methods such as qPCR and microarrays (Table 2). These methods are frequently used to select and measure target immune response gene sets and classic stress response genes (Table 2). RNA-seq and whole transcriptomic approaches represent a relatively novel approach to this area of research. RNA-seq has been demonstrated to have advantages compared to qPCR and microarrays (Wang *et al.* 2009; Garber *et al.* 2011). Some of these advantages include enabling the investigation of differential expression at a broader and more sensitive range, the discovery of new genes and transcribed regions, and identifying sequence variations such as SNP's and isoforms (Wang *et al.* 2009; Garber *et al.* 2011; Shiel *et al.* 2017). RNA-seq also does not require access to known sequence data, and so is well suited to the analysis of non-model organism transcriptomes (RNA actively transcribed/expressed from the genome in a specific cell or tissue) (Wang *et al.* 2009). RNA-seq can also reveal a snapshot of the presence and quantity of gene expression within an organism at a specific time (Chu and Corey 2012). Analysing the expressed genome can enable a detailed insight into functional mechanisms involved in responding to external stressors (Gracey *et al.* 2008; Zippay and Hofmann 2010). Analysis of gene expression signatures and patterns in susceptible and resilient molluscs in response to stressful conditions such as rapid temperature spikes can provide insight into molecular biomarkers of disease resistance. RNA-seq and the resulting gene expression signatures of different traits can then be used to identify simple sequence repeats and SNPs which can act as unique gene sequence identifiers and be used in molecular assisted breeding for desirable traits (Yang *et al.* 2011). Applying RNA-seq across multiple sampling points from the same individual enables powerful investigation of temporal gene expression profile fluctuations in a changing environment and can be used to identify the specific signatures and functional mechanisms that differ between groups of interest (Salvo *et al.* 2014; Fu *et al.* 2016; Bendjilali *et al.* 2017). Differential co-expression analysis (i.e. comparing groups of genes that have related or interacting expression patterns/functions) is also expected to provide greater accuracy and insight than standard expression analyses (de la Fuente 2010). One of the drawbacks with targeted approaches such as qPCR is that an important gene may still be expressed in the same relative level within disease susceptible individuals relative to resilient individuals, yet that gene may function differently based on mutations in the coding region. Furthermore, post-translational activities can affect the functional aspect of a gene product independent of the genes expression level. By investigating genes as networks, these complex differences unseen by targeted gene expression analysis can be identified (Zhao *et al.* 2010).

Genetic functional mechanism of summer mortality resilience and susceptibility

The genetic mechanisms responsible for summer mortality resilience can be investigated by determining the signatures of functional differences between susceptible and resilient molluscs. By measuring these differences during, after and prior to a heat stress event, a detailed system of basal, response and recovery functional mechanisms responsible for increased resilience could be identified. Studies comparing susceptible and resilient molluscs have been conducted primarily with bivalves, such as the Pacific oyster, *C. gigas* (Table 2), with more recent work on gastropods, such as *Haliotis laevigata* (Shiel *et al.* 2017). A meta-analysis of the literature shows that the function of genes differing between summer mortality resilient and susceptible animals depends on the tissue sampled (Table 2). Studies investigating the gonad tissue identified genes associated with reproduction that differ between susceptible and resilient families (Huvet *et al.* 2010; Fleury and Huvet 2012). Genes that differ between susceptible and resilient groups of animals could either be genes having a functional effect on resilience to summer mortality, or genes whose regulation is influenced in association with pathways associated with resilience.

Gene families associated with immune response and detoxification, such as the HSPs (particularly HSP70) may play a role in resilience. Heat shock proteins are common chaperone molecules that are produced during stress to prevent denaturation, assist folding and remove damaged proteins (Lindquist 1986; Parsell and Lindquist 1993). Due to this specific function, HSPs have been targeted in many summer mortality related studies as a marker of stress (Encomio and

Chu 2005; Cellura *et al.* 2006; Li *et al.* 2007a; Meistertzheim *et al.* 2009; Brokordt *et al.* 2015). However, due to the HSPs broad response to stress, studies defining the functional mechanisms associated with surviving summer mortality are still being explored. After exposure to *Vibrio* sp. bacterial infection and hypoxia, HSP70 production was significantly higher in susceptible *C. gigas* individuals, relative to resilient individuals (Samain *et al.* 2007). Fleury *et al.* (2010) also found that HSP70 was significantly up-regulated in summer mortality susceptible *C. gigas* oysters preceding and throughout a heat stress event. HSP70 was also suggested as markers of disease susceptibility in the Eastern oyster, *Crassostrea virginica* (Nikapitiya *et al.* 2014). These findings may suggest HSP70 expression may provide some cellular protection during these events. However, Lang *et al.* (2009), identified no difference in HSP70 expression between summer mortality high and low surviving *C. gigas* families when exposed to a heat stress event.

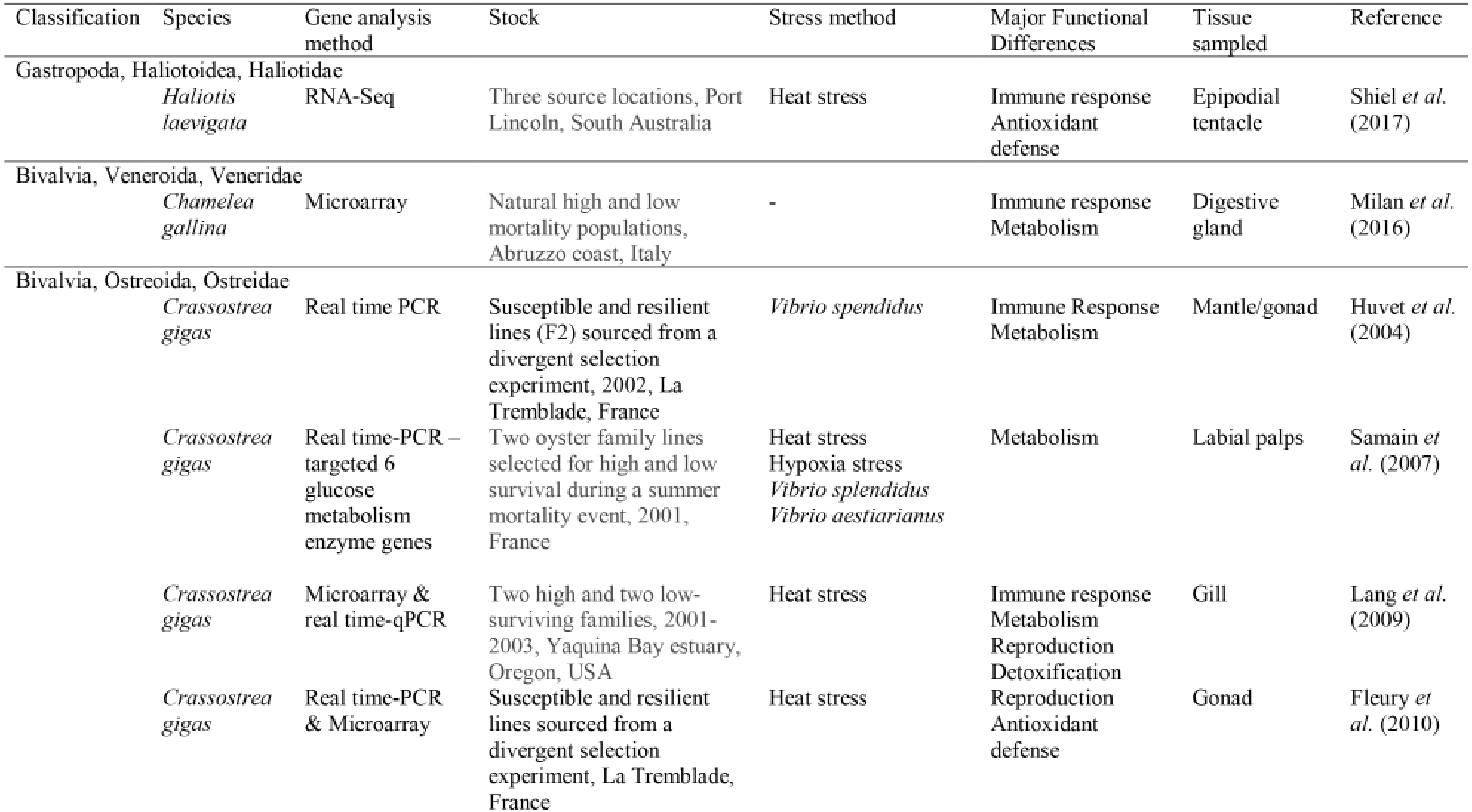
The heat shock response, particularly HSP expression is suggested to be accompanied by increasing metabolic related costs (Helms Cahan *et al.* 2017). Gene pathways associated with metabolism have been implicated in summer mortality survival, with an up-regulation of metabolism related gene expression pathways demonstrated in susceptible *C. gigas* (Huvet *et al.* 2004) and *C. virginica* (McDowell *et al.* 2014). One month prior to animal mortality, genes encoding for glucose metabolism enzymes have also been demonstrated to be lowly expressed in susceptible *C. gigas* infected with a *Vibrio* sp. bacterial infection, relative to resilient animals under the same conditions (Samain *et al.* 2007). Milan *et al.* (2016) also observed a dissimilarity in the metabolism related gene activities of the striped venus clam (*C. gallina)* from low and high summer mortality survival sites, and suggested that the susceptible population may possess reduced energy metabolism or lower feeding activity due to some bacterial or viral infection. Lang *et al.* (2009) suggested that opportunistic infection and cell damage maybe the result of metabolistic exhaustion caused by the elevated immune/stress response reaction in summer mortality susceptible families.

The response to reactive oxygen species (molecules that can promote oxidative stress in cells) has also been implicated in a defining characteristic of summer mortality resilience and susceptibility (Fleury *et al.* 2010; Samain 2011; Fleury and Huvet 2012). Higher expression of genes such as Catalase and Superoxide dismutase in resilient *C. gigas* and their ability to resist or detoxify oxidative species have been suggested to play a role in summer mortality resilience (Fleury *et al.* 2010; Fleury and Huvet 2012). Fleury *et al.* (2010) also linked the functional process of an increased antioxidant activity to the differences in metabolic and reproductive activity found between summer mortality resilient and susceptible lines. The immune response to pathogens and pathogen recognition is also an important area of investigation for defining summer mortality related resilience and susceptibility, however the intricacies involved in infection responses are complex. McDowell *et al.* (2014) identified several pathogen recognition genes such as cysteine-rich and C1qDC genes whose expression was aligned with disease resilience, however other pathogen recognition gene (Fibrinogen-related) expression was aligned with susceptibility, with the differences suggested to represent the complexities in response time or constitutive expression patterns between the susceptible and resilient groups. Further research is required to assess whether many infection related gene expression patterns whose expression differs between lines of varied disease resistant *C. gigas* could be interpreted either as just being a signal of an efficient antiviral response in these individuals, or as a genuine sign of their susceptibility to infectious replication (Nikapitiya *et al.* 2014; Segarra *et al.* 2014).

Identifying genetic signatures indicative of summer mortality resilience that can potentially predict the survival without subjecting animals to stress represents a vital area of research. Gene signatures present under non-stressed conditions may prepare the animal to tackle the stress, and such signatures are therefore known as “frontloading” (Barshis *et al.* 2013) or “preparative defense” (Dong *et al.* 2008) signatures . The genes involved are generally expressed at higher levels in stress resilient animals prior to a major stress event. This early high expression is suggested to act as a kind of buffer, allowing a more rapid response to the stress. Possible frontloading genes affecting summer mortality in *C. gigas* have been found to involve some immune response related genes (Fleury *et al.* 2010; Fleury and Huvet 2012; Rosa *et al.* 2012; Schmitt *et al.* 2013). Schmitt *et al.* (2013) utilised the gene expression signatures of a 42 gene immune related set, as well as sequence polymorphism to distinguish between *C. gigas* families with low and high summer mortality survival capacities. Twenty (11 and 9 highly expressed in the resilient and susceptible families, respectively) of these genes were differentially expressed between the two non-stimulated (not exposed to stress at the time) oyster families. In the only study investigating gastropods, Shiel *et al*. (2017) detected significantly higher gene expression of a gene with significant match to a transposon Ty3-G Gag Pol polyprotein, six months prior to a heat stress event, in resilient *H. laevigata* relative to susceptible. Interestingly, transposable elements have also been suggested to have a role in epigenetic and adaptive response to environment and stress (Slotkin and Martienssen 2007).

Down-regulated gene signatures in more resilient animals prior to a stress event are thought to enable the up-regulation of other vital stress response genes prior to or in response to a sudden stress event (Barshis et al. 2013). These types of gene signatures may explain the differences in summer mortality resilient and susceptible C. gigas prior to a heat stress event identified by (Fleury et al. 2010), who suggested that there may be some form of energetic trade-off occurring that reduces beneficial stress response processes in favour of reproductive processes.

Table 2. Gene expression studies comparing summer mortality resilient and susceptible mollusc families/lines or populations



Classification	Species	Gene analysis method	Stock	Stress method	Major Functional Differences	Tissue sampled	Reference
Gastropoda, Haliotoidea, Haliotidae							
	Haliotis laevigata	RNA-Seq	Three source locations, Port Lincoln, South Australia	Heat stress	Immune response Antioxidant defense	Epipodial tentacle	Shiel *et al.* (2017)
Bivalvia, Veneroida, Veneridae							
	Chamelea gallina	Microarray	Natural high and low mortality populations, Abruzzo coast, Italy	-	Immune response Metabolism	Digestive gland	Milan *et al.* (2016)
Bivalvia, Ostreoida, Ostreidae							
	Crassostrea gigas	Real time PCR	Susceptible and resilient lines (F2) sourced from a divergent selection experiment, 2002, La Tremblade, France	*Vibrio spendidus*	Immune Response Metabolism	Mantle/gonad	Huvet *et al.* (2004)
	Crassostrea gigas	Real time-PCR – targeted 6 glucose metabolism enzyme genes	Two oyster family lines selected for high and low survival during a summer mortality event, 2001, France	Heat stress Hypoxia stress *Vibrio splendidus* *Vibrio aestiarianus*	Metabolism	Labial palps	Samain *et al.* (2007)
	Crassostrea gigas	Microarray & real time-qPCR	Two high and two low-surviving families, 2001-2003, Yaquina Bay estuary, Oregon, USA	Heat stress	Immune response Metabolism Reproduction Detoxification	Gill	Lang *et al.* (2009)
	Crassostrea gigas	Real time-PCR & Microarray	Susceptible and resilient lines sourced from a divergent selection experiment, La Tremblade, France	Heat stress	Reproduction Antioxidant defense	Gonad	Fleury *et al.* (2010)

Crassostrea gigas	Microarray	Susceptible and resilient lines sourced from a divergent selection experiment, La Tremblade, France	Heat stress	Immune response Antioxidant defense	Gonad Gill Muscle	Fleury and Huvet (2012)
Crassostrea gigas	Digital gene expression (DGE) & qPCR	High and low infection surviving oysters sourced from Atlantic oyster farms, France	Bacterial pathogens *Vibrio splendidus* *Vibrio aestiarianus*	Immune response Genetic processing Metabolism Cytoskeleton reorganisation	Hemocytes	Rosa *et al.* (2012)
Crassostrea gigas	qPCR - 42 immune related gene set targeted approach	Two oyster lines selected for high and low survival during a summer mortality event, 2001, France	-	Immune response	Whole frozen (powder)	Schmitt *et al.* (2013)
Crassostrea gigas	Real time-PCR - 5 gene targeted approach	Two families of known differential susceptibility to OsHV-1, Marennes-Oléron Bay, France	Ostreid herpesvirus type 1 (OsHV-1)	Immune response	Mantle	Segarra *et al.* (2014)
Crassostrea virginica	RNA-seq	Two families of known differential susceptibility to the *Roseovarius* Oyster Disease, Rutgers University, NJ, USA	Bacterial pathogen *Rosevarius crassostreae*	Metabolism Detoxification Pathogen recognition Apoptosis Inflammation Extra cellular matrix remodelling	Whole body	McDowell *et al.* (2014)
Crassostrea virginica	qPCR-Targeted gene detection based on (McDowell *et al.*, 2014) gene set	Two families of known differential susceptibility to the *Roseovarius* Oyster Disease, Rutgers University, NJ, USA	Bacterial pathogens *Rosevarius crassostreae* and *Vibrio tubiashi* Protistan parasite *Perkinsus marinus*	Immune response	Whole body Haemolymph	Nikapitiya *et al.* (2014)

Application of genetic resources to combating summer mortality

One of the aims of searching for gene associations with summer mortality resilience is to identify the functional mechanism responsible for mortality and then use that knowledge to prevent such mortalities through selective breeding/genetic improvement programs and population management (Astorga 2014). Many of the improvements in the agriculture sector over the last several decades has been the result of genetically improving different breeds and varieties of livestock and crops. Comparatively, the investigation and development of genetic improvement programs for aquaculture-farmed species remains in its infancy. Gjedrem *et al.* (2012) estimated that only ~10% of the aquaculture production world-wide was the result of genetically improved stocks.

Marker assisted breeding programs can be developed to enhance summer mortality resilience in mollusc aquaculture through the identification of heritable SNP's associated with resilience. To combat summer mortality, these methods require in-depth screening and monitoring of traits and functional mechanisms potentially associated with resilience to summer mortality related stressors as well as the development of biological tests for enhancing resilience to such stressors. The evaluation of these features over multi-generational studies can reveal if the identified traits are heritable and correlations can be estimated within a breeding program to reveal the interrelationships between desirable traits such as growth and disease/stress tolerance. Genomic selection (Meuwissen *et al.* 2001), which utilises high coverage SNP data from the entire genome to estimate genomic breeding values and genomic relationships, might be well-suited to improving the genetics of difficult to measure quantitative traits such as summer mortality resilience. Developments in ultra-high throughput sequencing technologies for genotyping SNP's and/or differential gene expression analysis are making such approaches more feasible, and are now routinely applied to many livestock and fish selective breeding programs. The identification and enhancement of "fitter" genotypes will ultimately be essential for the sustainability of mollusc aquaculture and conservation efforts, safeguarding their economical and ecological value in the face of summer mortality related stressor, exacerbated by intensifying climate changes.

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