A. PHYSIOLOGY AND PATHOLOGY

Advancing Knowledge and Capabilities to Understand Coral Physiology and Pathology

Background

Signs abound in many areas that the natural plasticity of reef ecosystems to successfully respond to environmental (e.g., global climate change) and anthropogenic (e.g., land use, pollution) stressors has been exceeded. Vulnerable habitat conditions overlaid with multiple, concomitant stressors have compromised many coral communities to the point where their ability to rebound from natural disturbances is being lost. This is evidenced by the 27% loss of the world's coral reefs (Wilkinson 2002) and by predictions that estimate another 30% will be lost or impaired in less than 25 years. These predictions indicate that the current list of approximately 30-40 fatal diseases impacting corals will expand as will the frequency and extent of "coral bleaching" (Wilkinson 2002) resulting in effects ranging from reduced fitness, to community shifts, and ultimately to destruction of reef physical and biological functioning as we know it today.

Faced with many degrading environments over the planet, coral reefs are one of the key sentinels of ocean health and can serve as an indicator that links ocean and human health. Elevated disease levels among coral reefs serve as a sign of a sick ocean environment. With escalating disease reports throughout Pacific coral reefs, and predictions that point to a fate similar to that of Caribbean reefs, coral research and management are at a crossroads. This realization can instigate despair reflecting a hopeless inevitable fate that nothing can be done for reefs, or conversely be used as an instrument of change. It is our position that by developing an understanding of disease etiologies, causal links can be determined and factors driving these system failures can be identified. Developing such an understanding can move us from a triage mentality toward the ideal goal of *health management and preventative care* for coral reefs.

We are handicapped, however, in achieving preventative health care for coral reefs, in large part, because of a weak foundation in the basic sciences (e.g., biochemistry, cell biology, genetics, organismal and cellular physiology) of coral biology and the tools to enable rapid advancements. This has resulted in a fragmented research community, a menagerie of observations describing various coral afflictions with little coherence in how to make precise, defined observations in ways that promote comparative analysis, and almost no ability to discern mechanisms of disease.

The ability to successfully manage for healthy coral reefs depends on the inroads that are made into understanding the causes and effects of disease on coral vitality, i.e., coral pathology. Pathology however is rooted in the basic sciences of anatomy, physiology, microbiology, immunology, biochemistry and cell-molecular biology while integrating basic science with clinical applications. The very nature of pathology is predicated on the ability to discriminate between biological structures and functions occurring within a normal range and alterations resulting from disease processes. The depth at which we are

able to understand the normal structure and functions that govern corals at the colony level, individual, cellular and biochemical levels (i.e., their physiology) will dictate the speed and degree to which advancement is made in combating the spread of disease and ultimately proactively managing with the goal of healthy reefs.

Challenges and Recommendations

The Physiology & Pathology Working Group (PPWG) was tasked with identifying the information needed to advance knowledge and capabilities in coral physiology to better understand coral pathology. Thus providing a means to identify strategies to stop further reef degradation and create suitable conditions for natural restorative processes to take hold and flourish.

Coral biologists are challenged today with overcoming a void in information related to the functional processes of coral at cellular and organismal levels and the normal ranges in the functional parameters that define a healthy status, i.e., physiology. An adequate understanding of normal coral physiology and biochemistry is a prerequisite for building a sufficient foundation to competently study pathological conditions of corals. Understanding coral physiology and pathology requires defining the role of functional components at the cellular, systems, and organismal levels however the relationship between specific physiological sub-system (e.g., digestive, energy metabolism, nervous, reproductive, etc) processes, their regulation and the function of the whole animal has yet to be demonstrated for most coral species. This complicates discerning when or how normal biochemical/physiological processes have been disrupted to the extent that normal tolerance ranges of disease agents have been exceeded resulting in a pathogenic condition with lasting detrimental effects. Only when a full understanding of normal coral functions, as influenced by specific stressors, is achieved will the clinical manifestations of a specific disease be understood.

The pathogenesis and the etiology of a specific coral disease is partially known for only a few of the diseases described in the literature. Clinical manifestations have been described using a broad spectrum of biological/medical/veterinary terms that have been haphazardously applied to coral disease. The resulting nomenclature has painted a confusing picture that has led to misidentifying one syndrome for another. Another challenge for the coral disease community is to recognize the state of confusion within the field that can only be rectified by adopting standardized nomenclature and methodologies that will support exchange of information and ideas among coral disease investigators as well as with cross-disciplinary colleagues in fields not traditionally involved in coral disease research.

Pathogenesis: the pathologic, physiologic, or biochemical mechanism resulting in the development of a disease or morbid process

Etiology: the science and study of the causes of disease and their mode of operation

Clinical manifestations: gross morphological observations of corals

Compared to other wildlife diseases, coral disease research is in its infancy. Only recently have coral disease researchers begun applying technologies and methodologies routinely used in human and wildlife clinical and diagnostic medicine and pathology. Epidemiology is virtually nonexistent in the field. A growing number of scientists have begun applying biomedical approaches and adapting molecular biology tools in an effort to understand and characterize healthy corals and their responses when exposed to different stresses. These efforts show us the potential for understanding coral pathology and mechanisms for disease, and how a firmer grasp on this type of information can contribute to developing predictive indicators of adverse change in community health. However, there is a vast need to engage other researchers in the various aspects of coral health and disease. Persuading established researchers, in fields not traditionally part of marine science, to incorporate coral in their investigations or attracting new researchers to this field is difficult. The main challenges limiting progress in this arena include: 1) the availability of funding to conduct research on coral functional biology; 2) lack of a readily available source of research models; 3) few trained experts able to conduct the necessary research; and 4) lack of standardized field and laboratory approaches, including diagnostic criteria.

There is a critical need to equip scientists involved in coral research with the knowledge and skills to meet the challenges of health assessment and management. First and foremost, addressing information gaps on the functional biology of corals and their disease processes will require a broad integration of relevant disciplines that include health specialties (i.e., veterinary and medical science, pathology, medical microbiology, toxicology, epidemiology), marine scientists (i.e., wildlife and marine ecologists, marine biologists, oceanographers), basic scientists (i.e., biochemistry, cell physiology, microbiology, toxicology) and those who help interface with the public and politicians (i.e., resource managers, sociologists, economists). It is imperative to develop and provide advanced cross-disciplinary educational opportunities to encourage and equip the next generation of scientists to meet the challenges of coral reef health issues.

In addition to the strong support for strategic research in cellular physiology and funding routes pursued through directed funding by NOAA (i.e., grants program) and partnerships with NSF, EPA and other funding agencies, the PPWG recognized that a significant challenge to achieving success and a key underpinning is access to a valid research model (i.e., defined species, cell lines and zooxanthellae cultures). Agreement on selection of the model presents a significant challenge as many criteria need to be considered such as species range, growth forms, taxa with varying susceptibility to disease and bleaching, and known, reproducible genotypes. The PPWG also recognized the need to identify risk factors and preventative steps to reduce risks associated research activities (e.g., transport and introduction of pathogens via dive gear and tools, containment mechanisms to prevent the spread of coral disease under investigation in field and laboratory settings). Therefore, the PPWG devoted most of their time and effort in setting criteria for defining and selecting a model species for cellular physiological research which included identifying key information needs to support successful husbandry of the research animal model and identifying an initial set of candidate parameters to consider in delineating a normal physiological condition.

In the following section the PPWG identified six Strategic Objectives and associated Recommendations on practical approaches that can help address major gaps in the understanding of coral physiology and pathology. Achieving this goal will require instituting standard nomenclature to facilitate clear exchange of research and field observations, coral research models (species & cell lines) to elucidate physiological functions and morphological changes, and establishing standard culture conditions for consistency in use of model systems.

Strategic Objective A.1 **- Obtain strategic information needs in coral functional biology (e.g., cellular physiology, immunology, genetics, biochemistry)**.

Recommendation A.1.1: Provide targeted merit-based competitive grant opportunities to address knowledge gaps in the basic functional biology of corals through various Grants Programs offered by NOAA, NSF, EPA, NIEHS as well as private foundations.

There are limited sources of funding to conduct the research necessary to define physiological parameters and their natural variations in healthy coral. Most coral disease and health related funding has been targeted towards field monitoring that incorporates identification of gross lesions on coral to determine prevalence and incidence rates; microbiology to identify causative agents; histopathology to describe microscopic lesions; and a few biochemical and toxicological studies to measure responses of corals to various stressors, while few funding sources are specifically directed towards coral functional biology. The PPWG recommends establishing partnerships among granting agencies to develop a targeted RFP to support long term research and multi-investigator teams to determine baseline measures of coral health at the genetic, molecular, cellular, tissue and whole organismal level. This should include efforts to actively seek partnerships among the broader research community as a means of infusing new ideas and technologies from areas not traditionally considered as relevant to marine biology issues.

The PPWG identified five key information gaps that need to be addressed if the research and management community hopes to improve our understanding of coral pathology:

- Determine relationships between normal physiology and alterations caused by disease processes;
- Determine relationships between function at sub-system levels and functions at the whole organism and system levels;
- Elucidate how disruptions of normal physiological processes lead to pathologic processes;
- Determine the etiology, pathogenesis, and clinical manifestations of specific disease processes; and
- Predict clinical manifestations and appropriate treatment options for defined medical diagnoses.

By expanding research activities in areas of cellular physiology, genomics and proteomics, the research community will be better able to define nominal ranges of diagnostic parameters in healthy coral under normal spatial, temporal conditions and identify normal species-specific differences as a differential to recognize compromised health states. Through implementation of the recommendations put forward by this group, we can better characterize the complex mechanisms and factors underlying increases in bleaching events and coral disease outbreaks, as well as how human activities influence these processes. Understanding the mechanisms that confer resistance and susceptibility to disease, and deciphering the interactions between disease and environmental parameters will also provide the necessary information to support innovative development of diagnostic tools for rapid assessment of health and predictive capabilities of changes in health before disease signs manifest.

Strategic Objective A.2 **– Identify laboratory model(s) for coral research to enable rapid advances in our knowledge by focusing on fundamental biological concepts that are broadly applicable.**

Model species have been the key to rapid advances in disciplines such as developmental biology, genetics, toxicology, immunology, biochemistry and medicine. Model species have been developed in a number of taxa. Examples include *E. coli*, lambda phage, *Drosophila*, and *C. elegans* that have been instrumental in stimulating progress in our understanding of genetics and molecular biology. Selective breeding of species such as the brown rat and the common house mouse have produced white lab rats and mice that have been the workhorse of modern medicine. *Arabidopsis thaliana* (or Thale cress), *Medicago truncatula* (legume) and rice are three plant model species that have been essential for developing our understanding of the genetic and physiological bases responsible for fundamental biological functions that affect crop performance. Developmental genetics and cell biology have benefited enormously from studies of a non-mammalian vertebrate model, the zebrafish. Since its first recognition in the early 1970s, the zebrafish research community undertook several activities to promote uniform research conditions and open-exchange of information. Early on this included developing a manual for raising zebrafish for experiments and making it freely available and widely distributed among the research community. More recently, this free exchange of information has expanded to website resources and an enlarged zebrafish manual (see the following website for more information: http://zfin.org/zf_info/zfbook/zfbk.html), followed by the adoption of standard criterion for laboratory use of zebrafish. The website provides a large variety of resources in support of the zebrafish model, including products and supplies, gene collection, sequencing project, microarrays, funding opportunities, meeting information, and all types of document resources. The model is now listed on the NIH webpage for model organisms (Nih 2007) as one of eight non-mammalian models for biomedical research.

Our search for knowledge to date for hexacorals and octocorals has not been focused on a ‘model species’, but rather often represents the species readily available to a particular researcher. This has resulted in disparate studies involving hundreds of species or

subspecies, thus limiting the ability to compare data between studies and species. The PPWG recognized that all corals and their diseases are not the same, but an understanding of coral physiology requires focused development of one or two laboratory models that are most representative across scleractinian corals. There will always be a need to develop alternate models for specific diseases, but understanding basic coral physiology and the changes in these functions that result in disease will benefit from focused work on a few models.

Recommendation A.2.1 Establish criteria and select model species to focus basic coral physiological research.

Several suggestions for a cnidarian 'model species' have been published in the peer-reviewed literature, but only a few have recommended a scleractinian species. A brief summary of several recommended species and the disciplines for which they are most applicable are described in Appendix II and III. This literature review served to establish the currently available cnidarian models as well as to provide suggestions as to which criteria would be important in selecting a scleractinian model species for health and disease research.

Based on a review of the literature and the available expertise among working group members a list of criteria was developed for selecting a laboratory model for scleractinian coral physiology (see inset). The six possible candidates for the Indo-Pacific coral models identified by the PPWG are *Pocillopora damicornis, Stylophora pistillata, Porites rus, Galaxea fascicularis, Fungia scutaria, and Acropora formosa.* Each of these species has a different set of characteristics that make it a suitable candidate for a laboratory model for coral physiology studies. A brief rationale from published information is provided for each of these six species below.

Criteria for Selecting a Laboratory Model

1. Easy **adaptation** to long term captive rearing in closed, recirculation systems
2. Possible to provide many replicates through **fragmentation**
3. Widespread, geographical **distribution** in the Indo-Pacific
4. Reasonably **common**
5. Exhibits differences in **susceptibility** and **resistance** to disease
6. Representative of different **habitat** types (e.g., shallow water back reef and deeper water species)
7. Potential for **sexual reproduction** in captivity
8. Relatively **rapid** rates of **growth**
9. Branching and boulder growth **forms**

Pocillopora damicornis (aka, lace coral, cauliflower coral, bird's nest coral) is often referred to as the laboratory "white mouse" by coral biologists (Fig A.1). It is a major reef building coral widely distributed throughout the Indo-Pacific and Red Sea, and occurs in all shallow water habitats. It is affected by bleaching and disease worldwide, and has often served as an experimental subject for studies on coral physiology and reproduction. Its reproductive cycle is well described (Miller and Ayre 2004; Permata et

al. 2000; Richmond 1987; Sherman et al. 2006; Stoddart 1983; Ward 1992; Ward 1995; Whitaker 2006) and includes sexually produced planulae that are brooded to a fully developed Halcampoides-stage (Harrigan 1972). It is easily grown in a laboratory setting from fragments as well as larvae, and can be induced to produce planula year round by altering the night irradiance (Jokiel et al. 1985). The cryopreservation of P. damicornis larvae was reported by Hagedorn and colleagues (2006b). Its skeletal morphology, biochemical character and biomineralization process have been described (Brown et al. 1983; Domart-Coulon et al. 2004; Holden and Davis 2006; Letissier 1988; Tissier 1988; Vandermeulen 1975; Vandermeulen and Watabe 1973; Wainwright 1963). Conditions of stress and disease have been studied, including temperature extremes, bleaching, physical damage, sediment loading, ammonium enrichment, and infection by *Vibrio coralliilyticus* (Ben-Haim and Rosenberg 2002; Ben-Haim et al. 2003a; Ben-Haim et al. 2003b; D'croz and Mate 2004; Muller-Parker et al. 1994; Te 1992; Ward 1995). *P. damicornis* is susceptible to black-band disease (Dinsdale 2002; Willis et al. 2004), brown band disease (Willis et al. 2004), bacterial bleaching (Ben-Haim and Rosenberg 2002; Ben-Haim et al. 2003a; Ben-Haim et al. 2003b; Rosenberg and Ben-Haim 2002), mycelial fungal infections (Raghukumar and Raghukumar 1991) rapid tissue necrosis (RTN) (Luna et al. 2007), white syndrome (Willis et al. 2004) and skeletal eroding band disease (SEB) (Page and Willis 2008; Page et al. 2006; Willis et al. 2004). Primary cell cultures have been generated from *P. damicornis* and were demonstrated to produce aragonite crystals in adherent multicellular isolates (Domart-Coulon et al. 2001). Information is also available on growth characteristics following fragmentation, phylogenetic and symbiotic relationships, as well as histology and morphology. Recently the entire mitochondrial genome of *P. damicornis* was sequenced in a study designed to elucidate phylogenetically unique features of the family Pocilloporidae (Chen et al. 2008).

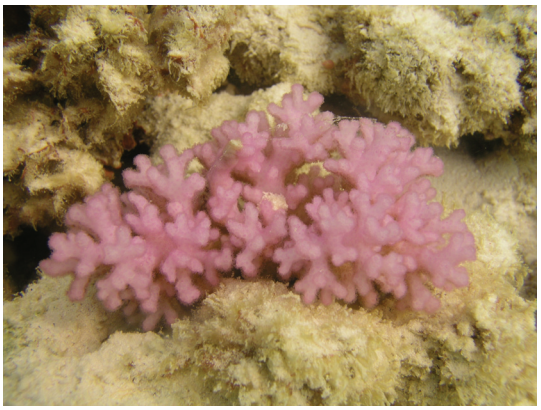

Figure A.1 ***Pocillopora damicornis*, photo by Greta Aeby.**

Stylophora pistillata (aka, false finger coral, cauliflower coral) is another well characterized scleractinian coral (Fig. A.2), whose widespread geographic distribution in the Indo-Pacific and commonality in shallow-water reef fringes make it an ideal candidate for a model species. It is a brooding species whose reproductive seasonality and lunar periodicity have been well described (Guest et al. 2005a; Guest et al. 2005b; Hall and Hughes 1996; Zakai et al. 2006). The larvae are easily induced to metamorphose in the laboratory (Baird and Morse 2004). *S. pistillata* has been used for many years as a key species for coral research in many fields, including coral biology, ecology, physiology, biochemistry, geochemistry, immunology, evolution, paleoecology, and biogeography (Loya, unpublished). Various features of the *S. pistillata* morphology

(Baird and Babcock 2000; Muscatine et al. 1997), physiology (Rinkevich and Loya 1986) and biochemistry (Dove et al. 2001; Dove et al. 1995; Richier et al. 2003; Rinkevich and Loya 1983; Tom et al. 1999; Zoccola et al. 2004; Zoccola et al. 1999) have been described, including characterization of the calcification process (Furla et al. 2000; Gattuso et al. 2000; Mass et al. 2007; Moya et al. 2006; Puverel et al. 2007; Puverel et al. 2005; Raz-Bahat et al. 2006) and dietary requirements (Houlbreque and Ferrier-Pages 2009; Houlbreque et al. 2003; Houlbrèque et al. 2004). The species has also been used in population level studies such as regional variations in population structure and dynamics, life history strategy, growth and regulation of populations, regeneration, competitive networks and reproductive strategy. Major contributions have been made on coral physiology, including insight into the symbiotic relationship between the coral host and its zooxanthellae, such as environmental effects on photosynthesis (Bhagooli and Hidaka 2003; Bhagooli and Hidaka 2004a; Ferrier-Pages et al. 2000), respiration (Hill and Ralph 2008; Leletkin 2005; Reynaud-Vaganay et al. 2001) and calcification mechanisms (Tambutte et al. 1996; Tambutte et al. 2007), energy budgets, carbon partitioning and utilization (Houlbrèque et al. 2004; Muscatine 1984; Reynaud et al. 2004), adaptive mechanisms of algal regulation and causes and effects of coral bleaching (Hoegh-Guldberg et al. 1987; Hueerkamp et al. 2001; Jones et al. 1999). The species has also been used to better understand obligatory, mutualistic or parasitic relationships and effects of marine pollution (crude oil, sewage and phosphates) at the population, individual and cellular levels (Loya et al. 2004; Rinkevich and Loya 1979; Walker and Ormond 1982). Recently the entire mitochondrial genome of *S. pistillata* was sequenced in a study designed to elucidate phylogenetically unique features of the family Pocilloporidae (Chen et al. 2008). *S. pistillata* is susceptible to Black-Band Disease (Dinsdale 2002; Willis et al. 2004), skeletal eroding band disease (SEB) (Page and Willis 2008; Willis et al. 2004) and Acroporid white syndrome (Roff et al. 2008; Willis et al. 2004).

Figure A.2 ***Stylophora pistillata*****, photo by Andy Bruckner.**

Porites rus (aka, plate and pillar coral) can form submassive, laminar branching, columnar structures, commonly over 5 meters across (Fig. A.3). It occurs throughout shallow reef environments in a wide variety of habitat types, where it may

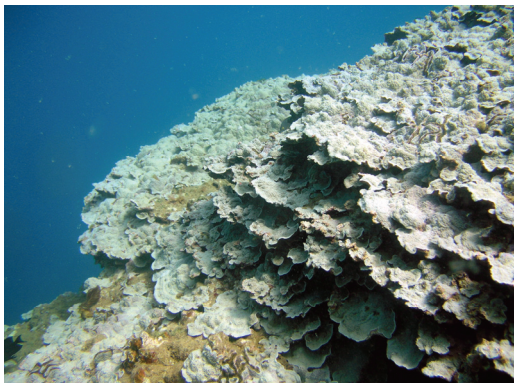

Figure A.3 ***Porites rus*****, photo by Greta Aeby**

be the dominant coral. Kolinski and Cox (2003) reviewed the modes and timing of gamete and planula release for Hawaiian scleractinian corals; they listed, but failed to provide information on *P. rus.* This coral exhibits high survivorship when exposed to anthropogenic stressors such as pollution and elevated temperatures and is fairly resistant to bleaching (Yap 2004; Yap and Molina 2003). It can rapidly colonize areas after disturbance, exhibits relatively rapid growth rates and can be readily propagated from fragments (Dizon and Yap 2006a). The Tahitian *P. rus* was shown to produce four MAAs (mycosporine-like amino acids) (Teai et al. 1997). The sea-floor spectral reflectance (R) is a characteristic utilized in remote sensing; *P. rus* exhibits a spectral reflectance pattern consistent with "blue" corals (Hochberg et al. 2004). *Porites rus* from Guam was shown to harbor the "C" phylo-type of *Symbiodinium-like* (Rodriguez-Lanetty 2003). Although the response was not real strong, *P. rus* did show antimicrobial activity against cyanobacteria in a study conducted by Koh (1997). *P. rus* was reported in 2003 as one of the six branching species of coral in the Indo-Pacific that exhibited signs of PUWS (Porites ulcerative white spot disease) (Raymundo et al. 2005; Raymundo et al. 2003). In addition, Work and co-workers (white paper, this report page 189) reported discoloration as a result of a sponge infestation in *P. rus* in American Samoa.

Galaxea fascicularis (aka, tooth coral, moon coral, galaxy coral) is a hermatypic coral with a gonochoric (distinct sexes) breeding system (Fig. A.4). *G. fascicularis* has been grown successfully in culture with a >200% weight increase over a 37 month period (Carlson 1999); although coral extension rates and calcification rates in some aquarium systems are close to those reported for natural reefs, anomalies have been observed such as decreased skeletal density and unusual changes in colony morphology (Clode and Marshall 2003b). This species can be difficult to maintain in an aquaria due to high light requirements and a high susceptibility to infections (brown jelly). Various features of the *G. fascicularis* morphology, physiology and biochemistry have been described in the literature: characterization of the mucus (Fung and Ding 1998; Fung et al. 1997); characterization of a GFP-like protein (Karasawa et al. 2003); calcification processes (Al-Horani et al. 2007; Al-Horani 2005; Al-Horani et al. 2005a; Al-Horani et al. 2005b; Clode and Marshall 2002; Marshall and Clode 2004a; Marshall and Clode 2004b; Marshall et al. 2007); corallite morphology (Crabbe and Smith 2006); stress studies (Bhagooli and Hidaka 2003; Bhagooli and Hidaka 2004b; Philipp and Fabricius 2003); egg proteins (Hayakawa et al. 2006; Hayakawa et al. 2005); skeletal matrix (Clode and Marshall 2003a; Fukuda et al. 2003); various genomic studies (Fukuda et al. 2002; Watanabe et al. 2005); characterization of their algal symbionts (Huang et al. 2006; Watanabe et al. 2006); dietary requirements (Houlbrèque et al. 2004); and micosporine-like amino acid (MAA) abundance

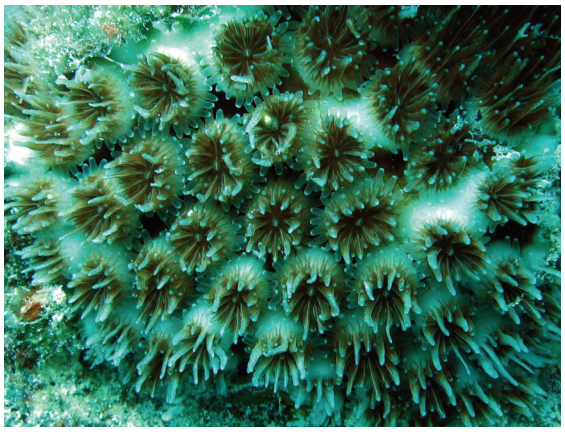

Figure A.4 *Galaxea fascicularis*, photo by Andy Bruckner.

(Yakovleva and Hidaka 2004). In an electrophoretic analysis, four soluble egg proteins were present in high abundance in the female egg, but were not found in the pseudo-eggs of functional males (Hayakawa et al. 2005). Gene expression, studied at the transcriptional level, was compared between female and functional male colonies. One of the vitellogenin-like proteins, GfEIP-4 protein, was cloned, sequenced and found to be expressed in both female functional eggs as well as male pseudo-eggs (Hayakawa et al. 2007). Although specific reports of disease in *G. fascicularis* have not been made, Winkler and colleagues (Winkler et al. 2004) reported SEB disease in coral reefs of Aqaba in the Red Sea including *Galaxea* sp. In addition, Work and co-workers (White paper, this report page 189) reported discoloration in *Galaxea* sp. in American Samoa.

Fungia scutaria (aka, mushroom coral) is the most common mushroom coral of the Indo-Pacific (Fig. A.5). It is free living and easy to collect. The species has separate sexes and releases eggs and sperm in the late afternoon, one or two days after a full moon. The larvae are azooxanthellate for 24 hours after fertilization and the process for establishing symbiosis can be observed without confounding background (Wood-Charlson et al. 2006). Krupp (1983) reported spawning to occur between 1700 and 1900 hours, 1-4 days following the full moon with only one short spawning event per lunar cycle. Krupp also reported that the oral pit formed by 24h and that the mouth became clearly visible by 39h; ingestion of zooxanthellae was not observed, but in a few days the planulae possessed zooxanthellae. *F. scutaria* also reproduce asexually (Krupp et al. 1993) and can regenerate from polyp stalks or from septal fragments (Krupp et al. 1996a). Their host-algal interactions have been studied, including means of infection and localization in tissues (Rodriguez-Lanetty et al. 2004; Rodriguez-Lanetty et al. 2006; Schwarz et al. 1999; Weis et al. 2001). Their sperm and planulae have been cryopreserved (Hagedorn et al. 2006a; Hagedorn et al. 2006b). The toxicity of the pesticide chlorpyrifos was tested against *F. scutaria's* gametes and planulae (Krupp et al. 1996b). From the examination of mucus samples for C,N,P composition (Krupp 1982) and their immunochemical nature (Krupp 1985), it was deduced that one of the components is sulfated acid polysaccharide and that the mucus was predominantly carbohydrate composition with some protein and of low nutritional quality. Although specific reports of disease in *F. scutaria* have not been made, Winkler and colleagues (Winkler et al. 2004) reported SEB disease in coral reefs of Aqaba in the Red Sea including *Fungia* sp.

Figure A.5 *Fungia scutaria*, photo by Thierry Work.

Acropora formosa (Dana 1846) (aka, staghorn coral) is a common branching coral that forms large thickets in shallow water on reef slopes, fringes, and lagoons (Fig. A.6); the species often coexists with other acroporids such as *A. nobilis* and *A. grandis* and is widely distributed throughout the Red Sea, the Pacific and Indian Oceans. This species reproduces sexually and can also be easily propagated from fragments (Okubo et al. 2005) but successful sexual reproduction following fragmentation is dependent upon the fragment size and the stage of oocyte development during fragmentation (Okubo et al. 2007). Staghorn-type *Acropora* sp. grows rapidly making it an ideal candidate for captive breeding/propagation. *A. formosa* calcification involves active Ca^{++} transport (Chalker 1976) and is impacted by ambient seawater temperature (Crossland 1984) with temperature exceeding light in determining growth and survival on a high-latitude reef. The development of scleractinian larvae from gamete-spawning coral, including *A. formosa*, was described in detail by Babcock and Heyward (1986) and in great detail for 5 related acroporid species (Okubo and Motokawa 2007). There are numerous papers describing the zooxanthellae associated with *A. formosa*: characterizing their DNA (Huang et al. 2006); describing their phosphate uptake (Jackson and Yellowlees 1990) and metabolism (Jackson et al. 1989); examining their potential for ammonia metabolism (Dudler and Miller 1988); and describing their turnover during bleaching (Jones and Yellowlees 1997). Toxic reactions to external chemicals both natural and anthropogenic have been reported: defensive chemicals exuded by soft corals can cause expulsion of *A. formosa*'s algae, followed by release of nematocysts and eventual tissue necrosis and death (Aceret et al. 1995) while herbicides have been shown to decrease the effectiveness of dinoflagellate photosynthesis by inhibiting the Photosystem II system (Jones and Kerswell 2003; Jones et al. 2003). *A. formosa* is susceptible to disease including Skeletal Eroding Band Disease (Page and Willis 2008; Riegl and Antonius 2003; Willis et al. 2004), brown band disease (Willis et al. 2004; Yarden et al. 2007), white syndrome (Willis et al. 2004), black-band disease (Willis et al. 2004) and skeletal anomalies (Peters et al. 1986; Sutherland et al. 2004; Work et al. 2008a). Photoprotection of *A. formosa* may be provided by both mycosporine-like amino acids, UV-absorbing compounds (Dunlap and Chalker 1986; Dunlap and Shick 1998) and by photopigments, one being a unique blue pigment in *A. formosa,* produced by the coral host (Dove et al. 2001; Dove et al. 1995). Another protective barrier, mucus, has been described in detail for *A. formosa*; the composition was reported to be polymers of proteoglycan crosslinked

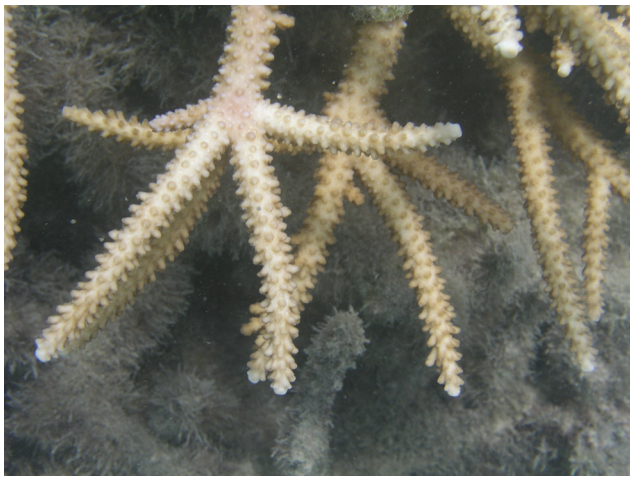

Figure A.6 *Acropora formosa*, photo by Greta Aeby.

by disulfides (Richards et al. 1983). Further characterization of the mucus side-chains indicated that they were sulfated oligosaccharides terminating with a mannitol (Meikle et al. 1987). In a comparative study, Meikle and colleagues found that there is not a common mucus structure for 6 different species of coral, rather the mucus was either dominated by unique protein or carbohydrate polymers (Meikle et al. 1988). Although complete genome information is not available for *A. formosa,* numerous studies have reported both genomic and mitochondrial DNA sequences.

Acropora millepora (a staghorn-type coral, no common name) is a hermatypic scleractinian coral with a digitate to branching growth form (Fig. A.7). It has a broad geographic distribution in the Indo-Pacific, found mainly in shallow water on reef slopes, fringes, and lagoons. *A. millepora* is a broadcast spawner that release eggs and sperm bundles once a year. Their embryology has been described in detail and the molecular cues have been investigated in parallel (Ball et al. 2002). They acquire their zooxanthellae during the planula larval stage (Van Oppen 2001). Ralph and co-workers demonstrated strong down-regulation of photosynthesis under conditions of high irradiance and observed little photoinhibitory damage to photosystem II (Ralph et al. 2002). High sea-surface temperatures lead to thermal stress in *A. millepora* with rapid bleaching, followed by recovery (with high retention of reproductive capacity) or death within 14 weeks; this in contrast to *Platygyra* sp. that resisted bleaching longer and took longer to recover (with loss of reproductive capacity) or die (Baird and Marshall 2002). Recently it was shown that there are shifts in the composition of the 'coral-associated bacterial assemblages' sampled from colonies of *A. millepora* during a natural bleaching event (Bourne et al. 2008a). The herbicide diuron did not inhibit fertilization in *A. millepora* but it did significantly impact metamorphosis in symbiont free larvae of this species (Negri et al. 2005). Other toxics, tributyltin, copper (Negri and Heyward 2001) and petroleum products (Negri and Heyward 2000), all inhibit *A. millepora* fertilization and metamorphosis to some extent. *A. millepora* is susceptible to black band disease (BBD) (Dinsdale 2002; Willis et al. 2004), skeletal eroding band disease SEB (Page and Willis 2008; Willis et al. 2004) and potentially skeletal growth anomalies (Work et al. 2008a). The innate immune capacity of *A. millepora* was characterized by scanning available EST and genomic resources; neither the Toll/TLR or the complement pathways were identified, but the presence of complement C3 and several MAC/PF are encouraging (Miller et al. 2007). Recently, the isolation and characterization of a mannose-binding lectin was reported; this pattern recognition protein binds bacterial pathogens as well as the coral symbiont

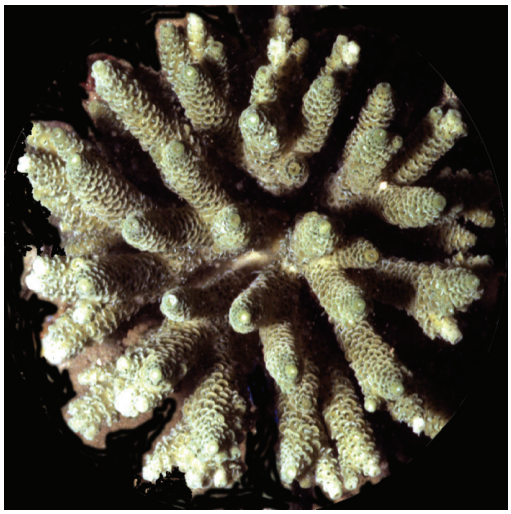

Figure A.7 ***Acropora millepora*****, photo by Andy Bruckner.**

Symbiodinium (Kvennefors et al. 2008). There are over 10,000 EST sequences listed in Genbank for *A. millepora* (Ball et al. 2002; Miller et al. 2007; Technau et al. 2005).

The working group submitted two candidate models for the Indo-Pacific region during the plenary discussion, ***P. damicornis*** and ***S. pistillata***. After additional discussion in the plenary session, the following two species were adopted as the recommendation of the Workshop, ***P. damicornis*** and ***A. millepora***

Strategic Objective A.3: Establish culture facilities to propagate corals, coral tissue and zooxanthellae cell cultures from the model species to augment laboratory studies.

Recommendation A.3.1: Determine the requirements and methodologies for establishing *in vitro* tissue culture and cell lines.

Coral tissue culture or zooxanthellae cell lines are not commercially available to researchers currently. The special requirements needed to establish and maintain coral cell cultures have not been fully elucidated and published such that this procedure is widely available and those reported successes of coral cell culture report only short term viability as primary cultures. There are however, private collections of zooxanthellae cell cultures, but only a handful of researchers are able to grow and maintain these cultures. The PPWG recommended the CDHC contact these investigators to assist in the development of a manual detailing the special requirements for establishing these cultures, and identify possible facilities that could maintain living cultures and provide them to researchers for a nominal cost, such as has been done in the harmful algal bloom (HAB's) community.

Several studies have undertaken the challenge to develop tissue/cell cultures of coral species. The importance of the presence of the extracellular matrix (ECM) was demonstrated by Schmid and colleagues (Schmid et al. 1999) as well as others (Dizon and Yap 2006a; Dizon and Yap 2006b; Lewis et al. 2006; Okubo et al. 2005; Permata and Hidaka 2005; Raymundo and Maypa 2004; Yap 2004). They noted that "when cultured, most cnidarian cells survive only when attached to ECM substrates; they rarely divide and die within short times." A review of the obstacles, approaches and improvements in culturing was published in 1999 (Schmid et al.) and updated in 2005 (Rinkevich). Moderate success was achieved in developing short-term primary cultures by several groups. Kopecky and Ostrander (1999) successfully cultured multicellular endothelial isolates from branching scleractinian coral (*Acropora micropthalma* and *P. damico*rnis) that survived in primary culture for 300h. In addition, five other species were successfully cultured: *Montipora digitata*, *S. pistillata*, and *Seriatopora hystri*x with *Porites sp.* less successfully. In a study conducted by Domart-Coulon et al. (2001) cells of apical coral colony fragments (*P. damicornis*) were isolated by spontaneous *in vitro* dissociation. Single dissociated cell types were separated by density in a discontinuous Percoll gradient. Primary cell cultures displayed a transient increase in alkaline phosphatase (ALP) activity, to the level observed in intact corals. Unique to this study was the demonstration of aragonite 'precipitation'. Continuous cell cultures of four

species of Octocorallia were reported by Frank et al. (2001) as well as a method to produce primary cell cultures for 10 cnidarian species including three Hexacorallia (*S. pistillata, Porites lutea, F. favus*). The primary cell cultures underwent cell proliferation within 2-3 weeks, and produced a collagenase soluble gelatinous matrix on the bottom of the wells. In another study focused on whole tissue isolation, soft tissue detachment from the skeleton of two branching coral species (*S. pistillata* and *P. damicornis*) yielded viable tissue capable of 70% survival for 3 days (Frank et al. 1994); these tissue pieces (containing whole polyps) quickly lost their morphology in dilute cell culture media, i.e., radial symmetry and oral-aboral polarity both were lost. After two days, in high glucose media, the tissue isolates dissociated layer by layer into individual cells, spreading in a circular outgrowth.

Recommendation A.3.2: Create the infrastructure and community-base to make experimental animals accessible and explore mechanisms to provide coral fragments from model species and in vitro tissue culture or cell lines to research community.

There are several groups interested in providing coral fragments or nubbins for coral research. The University of Miami's hatchery facility has been conducting feasibility studies with the goal of expanding their current culture capabilities (i.e., providing aplysia for neurological research) to coral production. However the major impediments, to establishing living stock collections of coral specimens of well defined genetic lines, are lack of funding and permit issues.

The PPWG identified one possible funding source to develop a coral culture facility. 'Living Stock Collections' is a potential source of short-term (36 months) support with the goal of developing innovative handling of stocks or well designed improvements in handling stocks. This is funded by the U.S. National Science Foundation (http://www.nsf.gov/pubs/2006/nsf06574/nsf06574.htm). There are only about 2-4 awards per year and the anticipated funding is limited with $1,000,000 available for all awards (new and renewed). Limitations of this program are: limited support for storage and distribution; no funds to conduct research beyond normal and appropriate curatorial efforts; and, no direct support for development of new reagents. Due to the short-term nature and limited support of this source of funding it is apparent that other resources must be explored.

To implement this recommendation, it will be necessary to obtain sustained government funding and/or assemble a team to actively identify benefactors from the private sector and secure long term funding until such a facility(ies) can be self-sustaining.

Recommendation A.3.3: Identify culture parameters that support normal physiologic condition and normal growth of the coral host model species.

The PPWG recommended the development of informational resources on coral husbandry, with emphasis on selected model species. These resources would describe optimal environmental parameters for culture facilities (e.g., light levels, water quality, food sources); identify potential stressors that may affect the system; develop protocols and methodologies for propagating corals through asexual and sexual reproduction with enhanced growth rates; and develop protocols for treating known diseases that affect aquarium invertebrates:

- Define tolerance ranges and optimum culture conditions (temperature, salinity, light intensity-wavelength, saltwater composition, density). Some information is presented in (Delbeek and Sprung 1994; Sprung and Delbeek 1997).
- Describe culture induced conditions, such as excess worms, macroalgae, parasites, and diseases, and provide protocols for treatment. See Borneman (2001) for more information.
- Define conditions required for sexual reproduction; provide protocols. See (Delbeek and Sprung 1994; Hagedorn et al. 2006a; Hagedorn et al. 2006b; Sprung and Delbeek 1997).
- Describe methods to promote optimum growth following fragmentation of coral. See (Ayre et al. 1997; Dizon and Yap 2006a; Okubo et al. 2005; Tarrant et al. 2004; Tsounis et al. 2006).

To implement this recommendation for developing these information products, the CDHC will need to identify experts in this field and assemble a team to draft the informational resources described above.

Recommendation A.3.4: Provide support to researchers in the optimal care and handling of the organisms by providing standard protocols for culturing the experimental animals through development of a manual for coral model laboratory organism.

The PPWG suggested that this manual could be patterned after the Zebrafish Manual. Once developed it should be published in hard copy and made available on the NOAA CDHC and CoRIS webpages. The group suggested the manual should include the following sections:

a. Introduction
b. Background [information on basic biology and biochemistry, nutritional requirements, physiological systems, reproductive characteristics, symbiotic relationships (algal and bacterial), susceptibility to stressors and genetics]
c. Distribution
d. Morphology
e. Developmental Biology
f. Molecular Biology (EST libraries, known gene sequences, phylogenetic relationships)
g. Husbandry

h. Availability and Processes for obtaining organism
i. Shipping instructions
j. Biosecurity
k. Permits, etc.
l. Histology: protocols and photographs
m. Standardized procedures for research studies
n. Research tools available (primers, specific antibodies, histological stains, etc.)

Creation of this manual is a team effort and will require experts to be identified who are willing to collaborate on the development of this manual. The CDHC could facilitate the team interactions by providing meeting logistics and publishing the manual after final peer-review.

Strategic Objective A.4: Determine key physiological parameters that typify a normal or healthy condition for the model coral species.

Recommendation A.4.1: Define a suite of physiological parameters that represents gross, cellular and subcellular levels of biological function and establish normal ranges for each parameter as criteria for determining health status of the model species.

The PPWG considered a number of possible assays that could be used to assess health status that include assays to assess photosynthetic potential, biochemical and cellular responses of coral to various stressors, regeneration of tissue, growth and reproduction. The group recognized that many of the specialized needs (e.g., immune function, cellular diagnostics, cell type and functional probes), would require a focused effort to develop the necessary assays. One approach to begin defining the physiological parameters suggested was to summarize methodologies and assays currently available as a resource manual for the model species. The initial list of factors to consider is as follows:

1. PAM fluorometer measurements, range and conditions, and standardized parameters for making the measurement;
2. Calcification rates under standard growing conditions;
3. Histology and morphology descriptions, and protocols for preparation of samples for light microscopy, SEM, TEM;
4. Microbial communities (culture-independent vs culture-dependent methods) under standard growing conditions.
5. Zooxanthellae symbionts characterized with respect to number, mitotic index, chlorophyll levels, and genotype;
6. Lesion development and regeneration;
7. Response to nutrient levels;
8. Developmental biology; and
9. Identification of cellular parameters useful in diagnosing environmental stressors and disease

The group recognized that much of the information proposed for inclusion in the resource described above may not be available, depending on the model species of interest. They identified seven key parameters that should be further explored in detail:

- Determine the specific ratio of protein-carbohydrate-fat in diet to support maximal growth.
- Determine nutritional requirements of the host, specifically any essential compounds, elements, vitamins, or trace elements required for successful metabolic homeostasis (i.e., nutrients provided by symbionts, mutualistic bacteria, etc that cannot be synthesized by the coral host) (Grover et al. 2002; Houlbreque and Ferrier-Pages 2008; Mills and Sebens 2004; Muscatine 1973; Muscatine and Hand 1958).
- Define the basics of the calcification process and required conditions for maximal growth. (Abramovitch-Gottlib et al. 2002; Al-Horani et al. 2005a; Elahi and Edmunds 2007; Fine and Tchernov 2007; Gattuso et al. 2000).
- Define the requirements to support normal reproduction and describe the developmental biology (Abramovitch-Gottlib et al. 2002; Lewis et al. 2006).
- Define specific metabolite levels or enzymatic activities describing critical cellular and tissue function and characteristics of microbial communities (TRFLP versus culture) under standard growing conditions. (Achermann 1980; Ball et al. 2002; De Jong et al. 2006; Gajewski et al. 1996; Holland 2004; Kopecky and Ostrander 1999; Miller and Harrison 1990; Seipel and Schmid 2006; Torras and Gonzalez-Crespo 2005; Watson and Mire 1999).

Recommendation A.4.2: Define markers of disease, both from a structural (e.g., histology) and functional (e.g., clinical diagnostic assays) perspective, that establish criteria for determining abnormal health condition in the coral host model species.

The PPWG identified four key research needs that could fill gaps in our understanding of changes in coral health, including morphological characterization using histology and electron microscopy; identification of cellular diagnostic parameters; characterization of patterns of lesion regeneration; and responses of the coral and associated symbionts to human and natural stressors:

- Reference materials (e.g., an atlas of coral tissue samples) with histological and morphological descriptions and photographs of healthy, stressed and diseased conditions in representative Pacific coral species. The Atlas should include protocols for preparation of samples for light microscopy, SEM, and TEM. (Ainsworth et al. 2006; Bourne et al. 2008a; Bourne et al. 2008b; Breitbart et al. 2005; Bythell et al. 2002; Gil-Agudelo et al. 2006; Klaus et al. 2007; Rosenberg et al. 2007; Work and Aeby 2006; Work et al. 2008a; Yokouchi et al. 2006).
- Characterize and describe cellular biomarkers for disease and develop a manual on assay and/or test protocols. (Downs and Downs 2007; Downs et al. 2005a; Downs et al. 2005b; Mc Clanahan et al. 2004; Peters 1984a; Peters 1984b; Peters 2001; Work and Aeby 2006; Work and Rameyer 2005).

- Understanding of conditions that support regeneration following lesion development (Hall 1997; Hall 2001; Henry and Hart 2005; Kramarsky-Winter 2004; Kramarsky-Winter and Loya 2000; Titlyanov et al. 2007).
- Characteristics of the response of the holobiont (coral host, zooxanthellae symbionts, and microbial community) to stressors (Branton et al. 1999; Hashimoto 2005; Lejeusne et al. 2006; Mc Dougall et al. 2006; Mitchelmore et al. 2007; Readman et al. 1996; Rougee et al. 2006).

Strategic Objective A.5: Create and support advanced educational opportunities.

Recommendation A.5.1: Develop an advanced degree program in coral pathology, offer continuing education in specialty topics for professionals and support fellowships for career development or cross-specialty training.

There is a critical need to build scientific capacity in the field of coral pathology and offer a health management perspective in resource management (Mullen et al. 2004; Sutherland et al. 2004; Woodley et al. 2008; Woodley et al. 2007; Work and Rameyer 2005). This will require a broad integration of relevant disciplines that assimilate expertise, tools and information from the coral research community as well as human, veterinary and wildlife scientists (e.g., pathologists, microbiologists, ecologists, cell physiologists). It is imperative to develop and provide advanced cross-disciplinary educational opportunities to encourage and equip the next generation of scientists to meet the challenges of coral reef health issues. This could include continuing education courses for professional development in histology/histopathology, environmental forensics, ecotoxicology, risk assessment and other disciplines. It should also include opportunities for advanced education such as a Master's program in coral pathology and graduate courses in cnidarian cell biology, histology and physiology.

Strategic Objective A.6: Organize a system of methodologies to investigate coral disease.

Recommendation A.6.1: Provide conceptual approaches to support sound science as coral biology merges with the field of medicine to understand disease causes and mechanisms that include guidance for the proper handling and containment regimes for laboratory and field experiments.

The PPWG discussed the concerns associated with the potential transfer of pathogenic organisms between locations and the lack of national or international guidelines for cleaning and disinfecting methods for vessels, equipment and divers that can prevent the transmission and/or introduction of pathogens to new hosts or locations. The potential for transmission may be elevated by researchers in direct contact with diseased corals or on reefs with disease outbreaks especially through 1) transfection experiments involving the removal of diseased tissue and transplantation to other presumably healthy hosts; 2) transfer of dive gear and tools that have not been decontaminated from a reef with a

disease outbreak to a neighboring reefs, or even to other locations within the same or different oceans; and 3) research to identify a causative agent via infection experiments conducted *in situ*. Other concerns include potential human health issues arising from the handling of infectious agents either in the field or laboratory.

A variety of groups have begun to develop protocols to protect against the introduction of pathogens or spread of disease including health certification for corals raised in laboratory settings and subsequently transplanted onto coral reefs, as well as protocols for cleaning and disinfecting vessels, dive gear and equipment prior to the transport between locations. Medical and veterinary containment measures may also be easily applied to potentially infectious disease outbreaks in the aquatic environment. It is recommended that a working group consider the available information and propose SOPs; an external review by a recognized authority of the SOPs is suggested to validate the process.

Physiology & Pathology Working Group Members:

Jo-Ann Leong (Chair) – Hawaii Inst. of Marine Biology, University of Hawaii, Kane'ohe, HI
Sylvia Galloway (Recorder) – NOAA NOS, Charleston, SC
Fenny Cox - NOAA NMFS, Pacific Islands Regional Office, Honolulu, HI
Marion Henry - Resource Management & Development, The Federated States of Micronesia
Margaret Miller - NOAA NMFS, Miami, FL
Mac Terzich – Pacific East Aquaculture, DelMarva Springs, MD
Stephen Victor - MS Research Department, Palau Int. Coral Reef Center, Koror, Palau
Esti Winter – Tel Aviv University, Tel Aviv, Israel
Gary Wobeser – University of Saskatchewan, Saskatoon, Saskatchewan, Canada