ADDRESSING OBSTICLES TO DEVELOPING OYSTER CULTURE IN HAWAIʻI

A THESIS SUBMITTTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT HILO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

TROPICAL CONSERVATION BIOLOGY & ENVIRONMENTAL SCIENCE

DECEMBER 2016

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Keywords: *Crassostrea gigas*, condition index, growth rates, *Polydora websteri*, fishponds, Molluscan Broodstock Program, Midori oysters, triploid, Hilo Bay.

Acknowledgments

I would like to sincerely thank the faculty and staff of the Pacific Aquaculture and Coastal Resources Center of the University of Hawai‘i at Hilo for their help and guidance during this project. I would especially like to thank Dr. Maria Haws for her mentoring and patience. I am also grateful for the help of Dr. Jason Adolf for excellent logical and statistical counsel, Dr. Armando Garcia-Ortega for his helpful advice and Dr. Marta J. DeMaintenon for her assistance identifying pests and parasites. In addition, I would like to give my thanks to Stephan Classen for his support and experience and to Kekai Edens, Jonathan Marusek, Brian Koval, and Sydney Gamiao for their countless hours assisting with data recording. Keoni Soares spent many hours collecting data. Ku‘uipo McCarty provided support and advice as well as the staff of Paepae O He‘eia Fishpond and Keawanui Fishpond for their collaboration. I also thank my research assistants Carli Hand, Austin Smith and Mike Pamatat for their assistance collecting data and performing experiments. I also thank Angela Beck for her superb editing and writing advice. This work was partially supported by the Hawai‘i Community Foundation on behalf of the Anderson Beck Kōkua a Ulu Fund, the Center for Tropical and Subtropical Aquaculture and the University of Hawaii Sea Grant College Program.

Abstract

A series of experiments was conducted on Oʻahu, Molokaʻi and Hawaiʻi Islands to examine specific factors that contribute to the feasibility of oyster culture for the state of Hawaiʻi. Two simultaneous studies were performed to measure the growth and condition index of five families of *Crassostrea gigas* (Pacific oyster) grown in the traditional Hawaiian fishponds of Heʻeia, Oʻahu, and Keawanui, Molokaʻi. Three of the oyster families (AA, AE, and EE) were produced by the Molluscan Broodstock Program (MBP) at Oregon State University. Two additional oyster varieties studied were the Midori strain of *C. gigas*, and a naturalized *C. gigas* strain found at Heʻeia fishpond called Oʻahu oysters. Oysters grew significantly faster in Keawanui than in Heʻeia fishpond ($P = 0.004$). There were significant differences in growth between the five families grown in Keawanui Fishpond ($P = 0.035$) but no significant difference was found between the same varieties grown at Heʻeia. Oysters grown in fishponds exhibited extremely high growth rates in excess of eight mm per month but suffered high mortality rates due to predation, biofouling and summer mortality. Midori oysters cultured in Hilo Bay, Hawaiʻi, had a significantly higher condition index than triploid or MBP oysters ($P = 0.002$). Regression analysis revealed that predation accounted for 57% of the variance in oyster mortality observed in Hilo Bay ($P < 0.0001$). Chlorophyll *a* was found to be a significant predictor for oyster growth, accounting for 40.8% of the variance in oyster growth in the Hilo Bay experiments ($P < 0.0001$). Analysis of various pest eradication treatments showed that the Super Salty Slush Puppy (SSSP) treatment adapted from Cox. et al. (2011) proved to be the most effective for removing the parasite *Polydora websteri* (oyster mudworm) from oysters without causing additional oyster mortality ($P < 0.0001$).

Table of Contents

List of Tables

List of Figures

Introduction

Aquaculture is the fastest-growing sector in all of food production around the world (Pogoda et al. 2011). Aquaculture provides a valuable source of food that compensates for depleting fish stocks, increasing human populations, and higher food demands. Hawai‘i’ currently consumes over 50 million pounds of seafood annually, of which 70% is imported (DLNR 2012). Much of the 750 miles coastline in Hawai‘i features protected bays suitable for producing a variety of aquaculture products (CRS Report 2006). There is great potential to increase local aquaculture in a culturally conscious manner by utilizing traditional Hawaiian fishponds (Keala et al. 2007, Sparks 1963).

Prior to Western contact, traditional Hawaiian fishponds were fundamental as a food source, producing an estimated two million pounds of seafood annually (Keala et al. 2007). In the 1960s and 1970s, a number of studies examined the potential for oyster culture in Hawai‘i, but concluded that more data on growth rates, survival, and condition index was needed (Sparks 1963, Brick 1970, Buttner & Karr 2009). Difficulties associated with predation, oyster mortality, and finding suitable locations, resulted in few successful ventures (Brick 1970, Howerton 2001). Currently, Hawai‘i industry has developed aquaculture for a wide variety of species including abalone, oysters, fish, and sea horses. Bivalve aquaculture is increasing worldwide due to high demand and market price (FAO 2014).

Oysters are filter feeders and are known to accumulate dangerous levels of fecal coliforms such as *Escherichia coli* and other harmful pathogens. Oyster aquaculture therefore requires careful water quality monitoring and testing by a certified lab to ensure health safety standards are achieved. A laboratory certified by a state health department official to monitor water quality is a legal requirement for shellfish growing areas in the United States. The Hawai‘i Department of Health (DOH) laboratory was certified in 2014 for shellfish sanitation purposes

and DOH began to monitor water quality in proposed shellfish growing areas for the first time in over 14 years. This opens the door for economic diversification and job growth in Hawai‘i.

Oyster culture in Hawai‘i may have an advantage over other states in the Pacific Northwest. Oyster aquaculture on the west coast of the United States has been seriously affected in recent years due to low levels of natural larval recruitment and high larvae mortalities in the hatcheries (Dumbauld et al. 2011, Busch et al. 2012). Both the low natural recruitment rates and hatchery failures are believed to be the result of ocean acidification due to higher atmospheric CO_2 levels (Barton et al. 2015). Long-term prospects for natural recruitment are bleak as increases in atmospheric carbon dioxide will likely continue to cause oceans worldwide to increase in acidity. This is projected to lead to drastic reductions in oyster larvae survival due to the breakdown of calcium carbonate in oyster shells (Kurihara et al. 2008). Located in the middle of the Pacific Ocean, Hawai‘i is the most isolated island chain in the world. Its waters are relatively stable and reports show no evidence of ocean acidification in marine waters (DOH Report 2014, Barton et al. 2015). This may make the waters highly suitable for large-scale oyster production and hatchery operations if current trends continue.

The Pacific Oyster <u>Crassostrea gigas</u> (Thunberg 1793)

This study focuses on the Pacific oyster, *Crassostrea gigas*, because it is the most commonly farmed aquaculture species in the world by weight and requires only simple aquaculture techniques (FAO 2010). *C. gigas* has an annual value of $4.2 billion and an annual harvest of 5.2 million metric tons, more than any other species of mollusk, fish or crustacean (FAO 2014). The oyster’s rapid growth and ability to adapt to a wide range of environmental conditions has made it suitable to be introduced and cultured in numerous countries around the world (Forrest et al. 2009). Surveys of ecosystem impacts of oyster cultivation have concluded

that any nominally adverse effects of commercial oyster cultivation like altered benthos may be offset by the largely positive effects such as increased fish and invertebrate production, ecosystem services and enhancement of biodiversity (Forrest et al. 2009).

C. gigas originated from Northeastern Asia and has been introduced into many other parts of the world including the Americas, Europe, and Africa. C. *gigas* constitutes 95% of total oyster production in European countries. Many of these countries have come to rely on C. *gigas* production more than their own native species of oyster because of its ease of propagation (Ruesink et al. 2005).

The *C. gigas* oyster was introduced to the Hawaiian Islands in the early 1920s to facilitate development of local bivalve aquaculture. Introductions occurred in 1926, 1928 and 1939 to Pearl Harbor and Kaneohe Bay, O‘ahu (Eldredge 1994). To date, *C. gigas* has not out-competed its native counterpart, *Dendostrea sandvicensis* (Hawaiian oyster), or become widespread (Coles et al.1997). This may be partly because *C. gigas* originated from temperate Asia and requires colder waters associated with winter to reach the high level of fecundity sufficient for effective spawning and reproduction. According to Ku‘uipo McCarty, Oyster Farm Manager at Kualoa Ranch in O‘ahu, Hawai‘i’s warm waters may also result in lower-quality oyster meat in diploid oysters especially during spawning season in August to September (McCarty, pers. comm. 2014, Dridi et al. 2007).

The development of new research and original techniques in aquaculture is essential to keeping pace with the increasing demand for seafood (Haws & Howerton 2011). There has been a large amount of research on *C. gigas* globally however, very few studies have been done in Hawai‘i. It is likely that *C. gigas* will experience significantly different growth rates in Hawai‘i than on the West Coast of the United States, since environmental conditions in the two regions

are different. Preliminary research has suggested that oysters grown in traditional Hawaiian fishponds can grow up to three times faster than in the other regions, but little scientific data currently exists (Haws et al. 2010, Haws, unpublished data).

Varieties of Crassostrea gigas

Shellfish culture often relies heavily on wild oyster seed collection in the early stages, but then transitions to use of hatchery production. The Pacific Oyster culture industry relies largely on hatchery production in the U. S. and other non-Asian countries where it was introduced. One advantage of hatchery production is that it allows for genetic selection to improve desirable traits such as growth rate, survival or appearance. A number of distinct strains, of *C. gigas* are grown for commercial production. Dr. Christopher Langdon at the Molluscan Broodstock Program (MBP) has bred *C. gigas* oysters for traits desirable for commercial production, such as increased growth rates and survival (Auyong 2009). Increases in desirable heritable traits by way of selective breeding of oysters have been documented worldwide (Rodrigues de Melo et al. 2016). Initial MPB breeding research focused on improving yields and survival by selecting the top 10% of best-performing broodstock for two generations (Auyong 2012). Selective breeding was conducted between individuals of unrelated families to ensure low coefficient of ancestry (Rodrigues de Melo et al. 2016). Selected lines called “Adams” and “Eves” showed 77% greater yields compared to wild control oysters (Auyong 2012). The wild oysters were from naturalized stocks sourced from Willapa Bay and Dabob Bay in Washington State and Pipestem Inlet in British Columbia, Canada (Rodrigues de Melo et al. 2016). Adam and Eve broodstock were crossed to produce Adam x Eve (AE), Adam x Adam (AA) and Eve x Eve (EE). These strains exhibit superior growth and survival in harsh environmental conditions compared to wild oysters (Auyong 2009). Another variety of *C. gigas* oyster used in commercial production is the Midori

oyster. The original stock was collected in near Midori River in southern Japan in 2004 by Dr. Langdon and bred in Oregon. This variety grows more slowly than typical *C. gigas* but may have increased tolerance to warm water environments and harsh conditions (Camara 2011). These experiments also utilized oysters produced by breeding oysters collected in Kaneohe Bay and believed to be the descendants of prior introductions. Its inclusion was based on the hypothesis that local adaptation may have occurred, making it suitable for culture because they have been surviving and reproducing in Hawaiian waters for generations.

Reproduction of Crassostrea gigas

C. gigas are protandrous hermaphrodites. This means that they are initially male, then change sex to female later in life, although some male do not change sex (Guo et al. 1998). *C. gigas* oysters spawn naturally in the late spring to mid-summer, when phytoplankton levels are high and the oysters' gonads have developed sufficiently due to the warmer water temperatures (FAO 2014). During the winter months oysters develop glycogen, which is converted to gametes as water temperatures rise in spring. Once the gametes have developed, oysters can spawn given specific environmental cues such as increased temperature, tidal fluctuations or increased phytoplankton levels. Most adult oysters begin spawning when water temperatures become greater than 20 ℃. Oysters release gametes into the water where they undergo external fertilization in the water column. After the initial spawning, oysters may spawn again approximately every four to six weeks from April through October (FAO 2014). They have very high fecundity resulting in large numbers of eggs, approximately 20 to 100 million, being released per oyster each spawn (Shatkin et al. 1997). *C. gigas* oysters are broadcast spawners which means most of the oysters in the area simultaneously release their eggs and sperm into the water column and they are subsequently fertilized (FAO 2014).

Triploid oysters

Numerous studies have found triploid oysters to have faster growth rates, higher disease resistance, and better meat quality than wild diploids (Nelson et al. 2004, Piferrer et al. 2009, FAO 2014). During the spring and summer months, diploid oysters expend large amounts of energy in reproduction and lose over half of their mass by expelling gametes into the water, substantially decreasing their food value, immune response, and growth rates (Nelson et al. 2004). Triploid oysters are not affected by the spawning season because they are generally non-reproductive. Instead, they reallocate their resources towards somatic tissue growth and deposition of glycogen. They continue to produce glycogen even during summer when glycogen is depleted in most diploid oysters (Allen et al. 1989). Research has shown that in harsh, low-food environments triploids accumulate 118% more glycogen, 93% more carbohydrates, and experience significantly reduced mortality compared to diploids, especially in tropical environments (Garnier-Gere et al. 2002).

Most studies have found higher meat mass, quality, and condition index (CI) in triploids than diploid oysters (Allen et al. 1989, Nell 2002, Garnier-Gere et al. 2002, Wang et al. 2002). Measurements of condition index is one of the primary methods used to assess health, overall commercial meat quality, table quality, and marketability of oysters (Pogoda et al. 2011). A great deal of global oyster output now focuses on triploid production (FAO 2014). Triploid oysters are crucial in aquaculture due to their increased growth rates, amplified immune response and superior all-season meat quality (Wang et al. 2002). While commercial interest in triploid oysters was slow to start, it has gained popularity, especially on the West Coast of North America where over 30% of Pacific oysters farmed in 2000 were triploid oysters. These figures are expected to increase in the future (Nelson et al. 2004).

Another advantage of triploid oysters is that they are relatively low-risk for culture in places where accidental release into the wild is a concern. Fertilization resulting from strip spawning male and female triploid oysters was only 0.0008% of that of regular diploids. The natural reproductive ability of triploids is presumed to be even less (Guo & Allen 1994a). In some rare cases, triploid oysters have been known to revert to being diploids. When this happens, they still retain much of their triploid-typical physiology but more often, triploids convert to a mosaic ploidy comprising diploid, triploid and tetraploid cells (Allen, S.K. 1994, Guo & Allen 1994a, Nell 2002).

Although conditions in the Hawaiian Islands may not be ideal for diploid oysters, triploid oysters may be well suited due to their comparatively high-quality meat and superior taste when grown in relatively warm waters (Wang et al. 2002). This makes triploids the logical choice for commercial and subsistence shellfish aquaculture in Hawaiian waters. Towards that end, an understanding of differential performance in diploids is central for triploid production since diploid parents' traits will contribute to the triploid performance. This is because selective breeding with diploids is the principle way to introduce new genetic material over time into the triploid lines (Allen, S.K. 1994, Guo & Allen 1994a).

Oyster feeding habits

Phytoplankton constitutes the primary food source for oysters (FAO 2014). Increases in food availability have been known to cause increases in shell length and meat weight (Brown and Hartwick 1988, Forrest et al. 2009, Grangeré et al. 2009). Oysters filter particles out of the water ranging from 4 microns to 100 microns in size and ingest nearly everything including detritus, phytoplankton, zooplankton, suspended benthic microalgae, and bacteria (Forrest et al. 2009). Prior studies established that adult *C. gigas* oysters filter up to 0.9 liters of water per

minute (Nelson et al. 2004). This filtering process increases water clarity, which has a positive effect on the abundance and growth of photosynthesizing marine organisms and benthic primary producers (Forrest et al. 2009). Research on oyster filtration has shown that oysters drastically reduce concentrations of total suspended solids (TSS), chlorophyll *a*, and carbon in their water (Ruesink et al. 2005). Oyster food availability is commonly measured by way of chlorophyll *a* concentration as a proxy for phytoplankton (Brown and Hartwick 1988, Nelson et al. 2004, Grangeré et al. 2009).

The Oyster Mudworm Polydora websteri

The oyster mudworm, *P. websteri*, is a marine polychaete that inhabits a variety of benthic substrate environments including coralline algae, corals, and mollusk shells (Blake 1996, Blake & Evans1973). *P. websteri* was first documented in the 1700s and was subsequently described as a pest in the 1800s after it destroyed oyster reefs in New Zealand (Blake & Evans 1973, Nell 2007, Read 2010). Of all the species of shell-boring polychaetes, *P. websteri* is the most damaging to commercial fisheries, aquaculture, and natural populations (Lafferty & Kuris 1996). *P. websteri* is the most prevalent parasite found in shallow-water bivalves in North America (Bergman *et al.* 1982, Blake & Evans 1973, Wargo & Ford 1993). Infestations have caused devastating consequences to mussel, scallop, abalone, and oyster fisheries around the world, and the worm has been implicated in the complete loss of oyster beds (Blake & Evans 1973, Nell 2007, Read 2010).

P. websteri larvae settle on a small indentation or crevice on the surface of the mollusk shell, commonly on the growing fringes near the mantle of the oyster, and bore into the shell (Handley & Bergquist 1997, Read 2010). They excavate their burrows by secreting an acid which dissolves the bivalve shell (Haigler 1969, Nell 2007). Infected oysters become stressed

physically and must divert valuable energy that would otherwise be used on growth and reproduction (Hooper 2001, Lafferty & Kuris 1996, Nel et al. 1996, Wargo & Ford 1993). In order to protect themselves from *P. websteri*, oysters react by secreting a chitinous conchiolin on the inside of the shell over the worm (Bailey-Brock & Ringwood 1982, Lafferty & Kuris 1996, Nell 2007, Wargo & Ford 1993). The oyster then secretes nacreous layers over the worm burrow, walling it off from the soft tissue and forming a blister, which the worm soon fills with mud, bacteria and pseudofeces (Bailey-Brock & Ringwood 1982, Wargo & Ford 1993).

P. websteri is a significant concern for oyster farmers for numerous reasons. In heavily infected oysters, there can be a multitude of burrows layered on top of each other, making the shell extremely porous and prone to breaking and crumbling (Bergman *et al.*, 1982, Haigler, 1969, Lafferty & Kuris 1996, Read 2010). The distorted, weak shells of infected oysters make them much more vulnerable to illness, predators and environmental stressors (Bergman et al. 1982, Lafferty & Kuris 1996, Read 2010). Infected oysters have decreased meat quality and shelf life, as well as an increased health risk if consumed raw (Bailey-Brock & Ringwood 1982). Wargo & Ford (1993) describe significantly reduced condition index (CI) as a result of heavy infestations in *C. virginica* on the East Coast of the United States. Infected oysters are not visually appealing due to mud blisters. If punctured during shucking, the blisters release a foul-smelling anoxic mud into the meat, and are thus not suitable for the more lucrative half-shell market (Gryder 2002). Burrows also pose a significant health hazard if pathogens such as *E. coli* or *Salmonella* are present in the waters in which they are growing (Bailey-Brock & Ringwood 1982).

P. websteri appears to be the most serious current threat to Hawai‘i’s developing oyster industry. Kualoa Ranch, the largest oyster farm in Hawai‘i, temporarily ceased operations for

several months in 2015 due to an infestation of *P. websteri*. Studies conducted by Bailey-Brock and Ringwood (1982) on O‘ahu Island found that unlike the established spring and summer reproductive season observed in temperate latitudes, *P. websteri* will reproduce year-round in tropical environments if food is plentiful. Hawaiian fishponds and estuaries provide the ideal year-round environmental conditions for oysters and worms alike, creating a high potential for *P. websteri* infestation in Hawai‘i (Bailey-Brock & Ringwood 1982).

Treatments for Polydora websteri

A variety of *P. websteri* control and removal treatments have been proposed including hyper- and hypo-saline solutions, chemical treatments such as bleach or acid, thermal shock treatments, and drying (Bailey-Brock & Ringwood 1982, Bishop & Hooper 2005, Nel et al. 1995). Hyper-saline treatments conducted by Bishop and Hooper (2005) consisting of 20-minute immersion in 290 g/L saline solution followed by drying for two hours resulted in a reduction in *P.websteri* prevalence from about 40% to 5% with no significant oyster mortality. However, Bailey-Brock and Ringwood (1982) found that hyper-saline treatments were only effective on oysters with undamaged shells, and caused oyster mortality if shells were cracked or damaged. In contrast, they concluded that hypo-saline treatments of six hours followed by drying for six hours caused extreme desiccation of *P. websteri* larvae and adults. They also noted that treatments reduced oyster growth rates, but did not increase oyster mortality. Similarly, Nel et al. (1996) determined that worms died in their burrows after six hours of fresh water exposure and recommended 12-hour treatments. Bailey-Brock and Ringwood (1982) found that using low concentrations (1-0.1%) of chlorine bleach and acetic acid was only effective at killing *P. websteri* larvae if combined with a drying period. Treatment with chlorine bleach and acetic acid resulted in high oyster mortality when concentrations were increased to 5% to kill worms in their

burrows. Studies investigating heat shock treatments consisting of submersion in heated sea water for 40 seconds at 70 °C resulted in significantly lower *P. websteri* infection (Nel et al. 1996). The treatment resulted in zero oyster mortality at 30 seconds, but when exposure time was lengthened to 45 seconds, mortality increased to 8.7% (Nel et al. 1996). Although this treatment requires careful timing to avoid oyster mortality, it has been shown to be more effective than 12 hours of fresh water treatment (Nel et al. 1996).

Controlling *P. websteri* infestations requires treatments which are highly efficacious for killing the mud worms while minimizing oyster mortality. It is also difficult to apply treatments that may be effective in the laboratory to a commercial oyster farming setting. Cox et al. (2012) experimented with a relatively new cold shock/hyper-saline treatment called the Super Salty Slush Puppy (SSSP) to control oyster overset and biofouling. Overset happens when small oysters attach to larger oysters grown for commercial cultivation, thereby decreasing the presentation value and competing with the primary oyster for food resources. Oysters were treated for 60-75 seconds in a solution of ice and salt at a concentration of 180-200 g/L NaCl and a temperature of -12 to -16°C, followed by reemission in culture water. The treatment was very effective at laboratory and commercial scales at controlling epibionts including barnacles, oyster overset, and flatworms (Cox et al. in 2012). Hyper-saline cold treatment may also be more practical and less time sensitive than dipping in hot water (Cox et al. 2012). Although *P. websteri* mortality was noted after cold treatment, effectiveness of the treatment on *P. websteri* was never investigated.

Challenges for Hawaiian oyster culture

Sparks (1963) conducted a survey of oyster potential of Hawai‘i and determined there was great potential for oyster culture in Hawaiian waters but concluded that studies on growth,

condition index, and survival were necessary before commercial plantings. Besides the general lack of scientific data on growth and marketability, one of the biggest problems documented in relation to oyster culture in Hawai'i is predation and pest control. Pest control and predation is also one of the most widespread concerns associated with oyster culture on a global basis.

A study conducted Brick (1970) by in Kaneohe Bay, Hawai'i had intended to analyze the growth rates and condition index (CI) of *Crassostrea virginica* (eastern oyster) and *C. gigas* to establishing a culture program in Hawai'i. Only six weeks into the study, Brick (1970) found all the oysters were dead, apparently killed by the polychaete flatworm *Stylochus,* commonly known as the oyster leach. A second trial at the same and various other locations around Kaneohe Bay also resulted in widespread oyster mortality due to *Stylochus* predation. Other research has shown that oysters grown in Hawai'i have been preyed upon by boring sponges, *Stylochus* flatworms, crabs, snails, and polychaetes such as *Polydora websteri*, and can become encrusted with epiphytic bryozoans, barnacles, sponges, and tunicates (Sparks 1963, Bailey-Brock & Ringwood 1982). Other studies have found that oysters may not reach sufficient commercial quality due to the lack of food availability and year-round warm waters normally associated with the natural spawning season (Haws unpub. data). Therefore, this study focused on these issues (growth, condition index and predation) and how they specifically relate to oyster culture in Hawai'i.

Study Objectives and Rationales

Growth, condition index and chlorophyll a as an indicator for farm site selection

Despite problems associated with oyster culture in Hawaiian fishponds, there is a strong demand for locally-grown high-quality oysters (McCarty, pers. comm. 2014). Commercial oyster farming is new to Hawai'i and is currently solely conducted in traditional Hawaiian fishponds,

Hilo Bay, and a modified golf course pond on west side of Hawai‘i Island. A few oyster growth trials have been conducted in Hawaiian fishponds (Brick 1970, Haws unpub. data), but oyster survival and growth have not been quantified or compared with growth rates in open water areas in Hawai‘i such as Hilo Bay. Hence, the specific goals for this project were to quantify and compare growth rates and meat quality (CI) and assess causes of mortality of several varieties of MBP, triploid, Midori, and O‘ahu *C. gigas* oysters grown in He‘eia Fishpond, O‘ahu, Keawanui Fishpond, Moloka‘i, and Hilo Bay, Hawai‘i. The data from several oyster growth and CI studies conducted over the course of a three-year period was compiled and presented the information for the first time in a comprehensive assessment of oyster culture potential for the state of Hawai‘i. Using chlorophyll *a*, and dissolved oxygen (DO) data from the Pacific Islands Ocean Observing System (PacIOOS) Water Quality Buoy, this study preformed correlations with oyster growth data. These studies were compared with similar studies done Hawai‘i and research from the West Coast of the United States and around the world in order to determine Hawai‘i’s oyster culture potential.

Predation and mortality

Oyster mortality, whether due to predation, summer mortality, or disease outbreak, is a serious concern for the oyster industry worldwide (Mondol et al. 2016). Predation is a ubiquitous problem in nearly all oyster-farming areas around the world. This study was designed to assess these problems and provide the most practical solutions to facilitate further development of bivalve aquaculture in Hawai‘i. An improved understanding of predation and parasites, including possible treatments, is fundamental to ensuring the success of oyster farming in Hawai‘i during its current early stage of industry development. About 50% mortality is expected throughout the duration of the grow-out period. Larger mortality events can lead to complete cessation of

farming and job loss. Thus, assessing rates and sources of mortality is fundamental to determining whether oyster culture is feasible and profitable in the state of Hawai‘i. One of the chief goals of this research was to develop a comprehensive treatment program for *P. websteri* and other pests in a cost effective and practical manner.

Hypotheses

H_1 : MBP oysters have faster growth rates in fishponds than Midori or O‘ahu oysters.

H_2 : Triploid oysters have faster growth rates than MBP or Midori oysters in Hilo Bay.

H_3 : Triploid oysters have higher CI than MBP or Midori oysters in Hilo Bay.

H_4 : Chlorophyll *a* is a significant predictor for oyster growth.

H_5 : The SSSP is the most effective treatment at *P. websteri* removal.

Materials and Methods

Growth trials were conducted on three of the main Hawaiian Islands. This study utilized standardized equipment and evaluation criteria at study sites.

Fishpond trials

Growth, CI and survival was examined for five families of *C. gigas* oysters simultaneously grown in two traditional Hawaiian fishponds. The study was done over the period of 12-months starting October 1st, 2011, and ending September 20th, 2012. He‘eia Fishpond is located on the northeast side of the island of O‘ahu at 21° 26’ 14’’ N and 157° 48’ 33’’ W and is 88.4 acres in size (Fig. 1), (Keala et al. 2007). Keawanui Fishpond is situated on the southeast side of the island of Moloka‘i at 21° 03’13’’ N and 156° 50’ 58’’ W and covers an area of about 72 acres (EPA Report 2003). Both fishponds are characterized by shallow waters of around one meter in depth with occasional shallow and deeper patches. The fishponds are enclosed by rock walls with sluice gates that regulate water flow in and out of the fishponds. Salinity and other water parameters can vary widely depending on local weather and season.

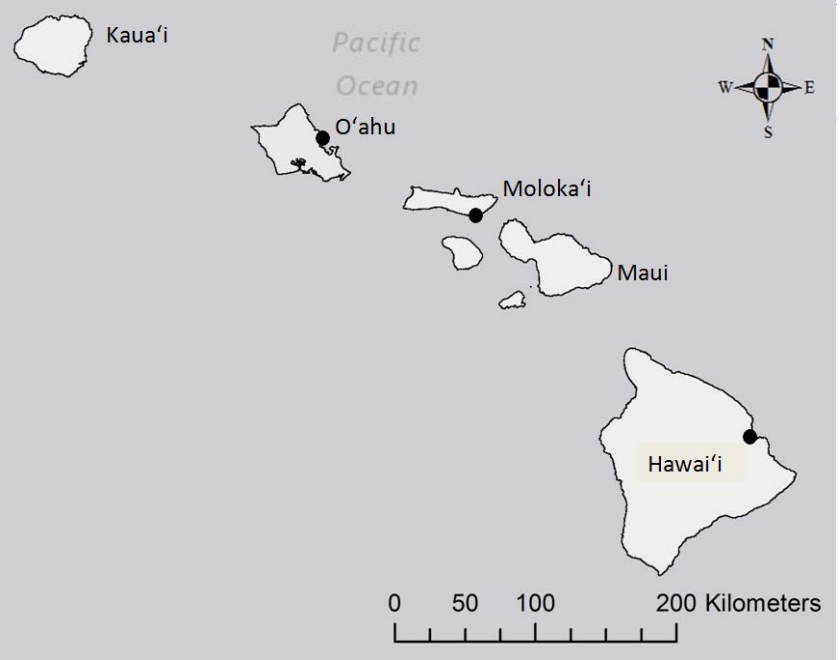

Figure 1. Study locations He'eia Fishpond, O'ahu, Keawanui Fishpond, Moloka'i, and Hilo Bay, Hawai'i, shown on map. Map created with GIS

Three MBP strains (AE, AA, and EE) and two alternative strains (Midori & O'ahu) were grown in both fishponds concurrently. Oysters were grown in cages suspended on a horizontal long line in the fishponds. The cages were suspended approximately at the mid-point of the water column. Oyster cages were visited monthly to measure oysters, clean cages, shuffle cage position, and remove empty shells. There were four replicate cages for each family of oysters with initial starting densities of 500 oysters per cage and initial shell length of 10 ± 5 mm. Thirty oysters from each cage were measured as a proxy for growth during each sampling period resulting in 120 oysters measured per family per sample period. Cages were randomly reassigned positions on the line after each inspection to eliminate any potential effect from the position on the line. Growth was determined by subtracting initial mean shell length from final mean shell length over the period analyzed.

Hilo Bay trials

Hilo Bay is typically autotrophic with high primary productivity interspersed with the occasional high river flow conditions. These high-flow conditions have been shown to cause large amounts of flushing and may lead to phytoplankton crashes (Mead et al. 2010, Weigner et al. 2010). Successful oyster culture that maximizes growth and minimizes mortality requires a location that is well-flushed and has freshwater input, abundant phytoplankton, and an optimal depth and temperature profile (Cassis et al. 2011, Pogoda et al. 2011, Wallace et al. 2008). Hilo Bay is one of many bays and harbors in the state of Hawai‘i that facilitate nearly year-round phytoplankton blooms. Hilo Bay is a large bay on the east side of Hawai‘i Island (Fig. 1). Three rivers, Wailoa, Wailuku, and Honoli‘i, empty into the bay. The two larger rivers, the Wailoa and the Wailuku, flow into Hilo Bay at an average combined rate of 2.8 million cubic meters per day (Wiegner & Mead 2009). Submarine groundwater discharge from underwater freshwater springs is also responsible for a significant amount of freshwater input into Hilo Bay (Mead & Wiegner 2010). These fresh water inputs introduce nutrients into the bay that support the growth of phytoplankton on which oysters feed.

Two study sites were chosen in Hilo Bay. Site selection was based on protection from wave action, distance from boat traffic, and high levels of phytoplankton. Water quality parameters were sampled at both sites. Samples were collected at a depth of 4 m at each site on three different days during November 2011. Heavy rains were experienced on one sampling day. After water samples were collected, DO, salinity, and pH were measured with an YSI 85 multi-parameter probe and Thermo Orion 230A+ portable pH/ISE meter, respectively.

Oysters were deployed at study site 1 on September 28th, 2012. Study site 1, located at 19° 44’ 13” N, 155° 04’ 52” W, was selected because of its distance from boat traffic, its close

proximity to a water quality buoy, and its position within a nutrient plume originating from the Wailuku River and traveling north parallel to the coastline (Weigner & Mead 2009, Weigner et al. 2011). Three cages of diploids and three cages of triploids were hung from a horizontal long-line positioned at three m depth, with the cages suspended one m below the line. This depth was chosen because previous research found that oysters grown at a depth of four m had greater final volumes than at other depths (Cassis et al. 2011). At the start of the experiment, the spat were sieved and graded to be approximately 4 mm in size with 1000 oysters per cage. Cages were opened every two weeks to take growth measurements and assess survival and predation. The oysters and cages were also cleaned at this time. The position of each cage on the long-line was changed by rotation every two weeks to control for confounding factors such as small differences in the culture environment that might affect growth. The number and types of pests were noted along with the severity of biofouling. Average growth was recorded by selecting 30 oysters at random from each cage and measuring length, width, and depth.

The concentration of chlorophyll *a* $\mu g/L^{-1}$, and dissolved oxygen (DO) mg/L^{-1} was monitored by the PacIOOS Water Quality Buoy (Fig. 2). The water quality buoy provided continuous data on a variety of parameters including chlorophyll, DO, pH, temperature, and salinity to the public sampled at 1 meter depth. The buoy was placed intentionally in the nutrient plume by Dr. Jason Adolf, Department of Marine Sciences, and University of Hawai‘i at Hilo, who manages and maintains the buoy. For all water quality parameters, the data was averaged to provide a mean numeric value for the parameter over the preceding two weeks before the growth measurement. Similarly, the mean change in shell length per two-week interval was calculated by deducting the last measurement from the most recent. This resulted in numeric value in mm for the average growth of the oyster over a two-week time span. A regression analysis was

preformed to test whether higher phytoplankton concentration is a predictor for a higher rate of shell growth.

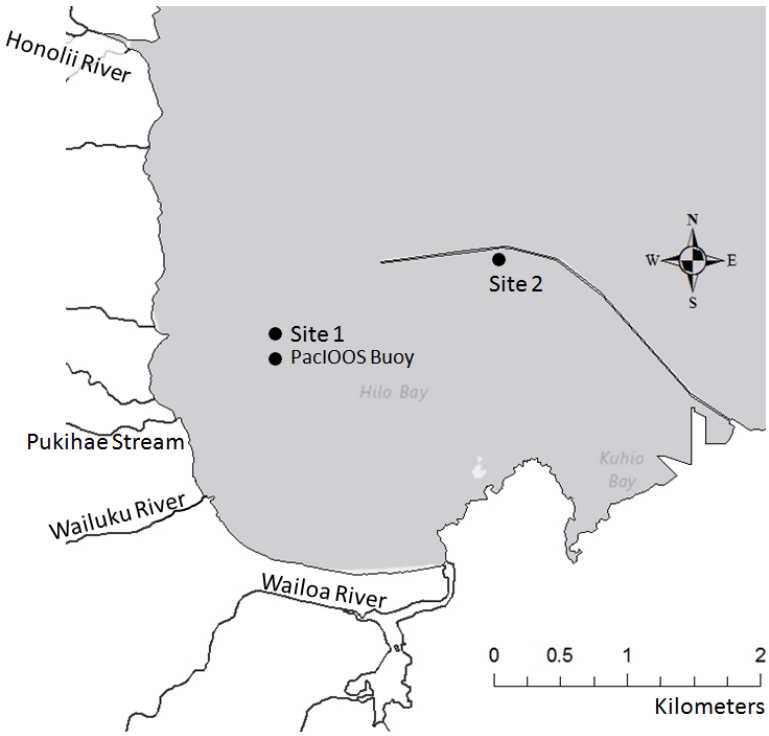

Figure 2. Map of Hilo Bay with study locations. Hilo, HI, USA. Map created with GIS.

The second study, located at site 2 (Fig. 2), started December 5, 2014, and ended February 22, 2016. The study site, located at 19° 44’ 34” N, 115° 3’ 54” W, was selected because of its distance from boat traffic and the protection from waves provided by the Hilo Bay Breakwall. Three oyster cages were hung from a horizontal 10 m long-line positioned at three m depth, with the cages suspended one meter below the line. One cage contained triploids, one cage contained Midoris, and one cage contained a mix of MBP strains. Cages were visited every two weeks and the same sampling and observation methodology as Site 1 was used. Oyster mortality was recorded for each cage. A Kruskal-Wallis test was used to compare the average growth rates and CI of Midori, MBP and triploid oysters.

Condition index

In bivalve aquaculture, the CI is most commonly calculated using the Davenport & Chen (1987) formula:

$$CI = [\text{dry meat mass (g)} \times 100]/\text{dry shell mass (g)}$$

For this study, 30 oysters were taken at random from each cage at the end of the grow-out period. The oysters were cleaned and shucked. Oysters were placed in a drying oven for 24 hours at 60 °C. The dry weight of the meat and shell was measured and the CI was calculated. This method of determining CI maximizes accuracy and precision because it uses the dry weight of the shell instead of the highly variable weight of the body cavity of the oyster (Pogoda et al. 2011).

Removal of <u>Polydora websteri</u>

Oysters infected with *P. websteri* were obtained from Kualoa Ranch on O‘ahu and air-freighted to the Pacific Aquaculture Resources Center (PACRC) on Hawai‘i Island for analysis. Oysters were held in isolated tanks and fed a diet of *Isochrysis* and *Chaetocerous* microalgae. Water temperature was held at 24° C. The mean size of the experimental oysters was 3-4 cm with *P. websteri* infection levels of 20-100 worms per oyster.

Five treatments and an untreated control were designed to incorporate some of the most effective techniques for *P. websteri* removal documented in literature (Fig. 3). The goal was to find the most effective combination of treatments while minimizing oyster mortality. Oysters were randomly selected from infected stock with 30 oysters per treatment. Industrial rock salt was used for hyper-saline solutions because it is readily available and relatively inexpensive. Oysters were dried passively at room temperature for any required drying during treatment. For each treatment, the selected oysters were placed in a single mesh bag and treated in a 20 L

bucket, then transferred to a 40 L tank at 24 °C with no water flow and light aeration for 24 hours before analysis of treatment effectiveness. Control oysters were allowed to sit in sea water for three hours and then transferred directly to a 40 L tank. Twenty four hours after treatment, the total number of *P. websteri* worms that had exited the shells were counted. Shells were broken with a hammer and examined under a dissecting microscope to count *P. websteri* still in the shells. After the *P. websteri* count, oysters from each treatment were placed back in their 40 L tanks and allowed to resume feeding for five days. Oyster mortality was recorded for each treatment.

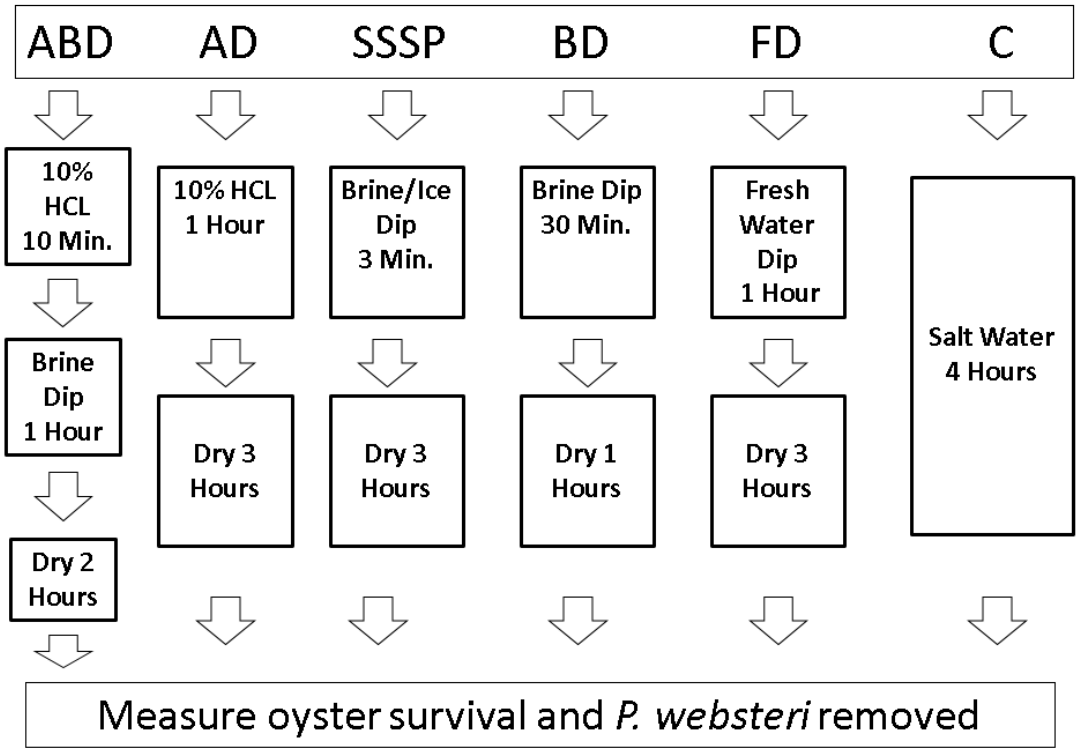

Figure 3. Flowchart of *P. websteri* removal treatments ABD, AD, SSSP, BD, FD, and C.

Treatments for P. websteri

1) Three percent muriatic acid bath in fresh water for 10 minutes followed by brine dip (250 g/L NaCl) for 1 hour followed by drying for 2 hours (ABD).

2) Three percent muriatic acid bath in fresh water for 1 hour followed by drying for 3 hours (AD).
3) Submersion in brine (250 g/L NaCl) chilled with ice to -10 to -30 °C for 2 minutes followed by drying for 3 hours (SSSP). This "Super Salty Slush Puppy" treatment was adapted from Cox et al. (2012).
4) Submersion in brine (250 g/L NaCl) for 30 minutes followed by drying for 1 hour (BD). Adapted from Bishop and Hooper (2005).
5) Submersion in fresh water for 1 hour followed by drying for 3 hours (FD). Adapted from Bailey-Brock and Ringwood (1982).
6) Control (C) no treatment.

Results

Oyster growth and survival in Hawaiian fishponds

Analysis of the mean shell growth of oysters from each fishpond with a two-sample *t*-test shows that oysters grown in Keawanui Fishpond grew significantly faster than oysters in He‘eia Fishpond (Fig. 4, $P = 0.004$). The mean increase in shell length for all families in each fishpond was 58.6 ± 2.4 mm and 64.1 ± 5.9 mm over 232 days for He‘eia and Keawanui Fishpond, respectively. The mean growth rate was approximately 7.5 ± 2.7 mm per month for He‘eia and 8.7 ± 1.4 mm per month for Keawanui. Although the mean growth was higher in Keawanui Fishpond, standard deviation was also higher, representing less consistent growth rates than He‘eia Fishpond. Oyster mortality was observed during every sampling period and by day 232 over 50% of the oysters had died in both fishponds. A large mortality event occurred nearly simultaneously in both He‘eia and Keawanui Fishponds between July and August 2012, killing over 75% of the remaining stock. Oysters with a length greater than 60 mm were most severely

impacted. The magnitude of the mortality event had a disproportionate effect on larger oysters, therefore only the growth data prior to the mortality event (the first 232 days of grow-out) was used to determine the mean increase in shell length of the population.

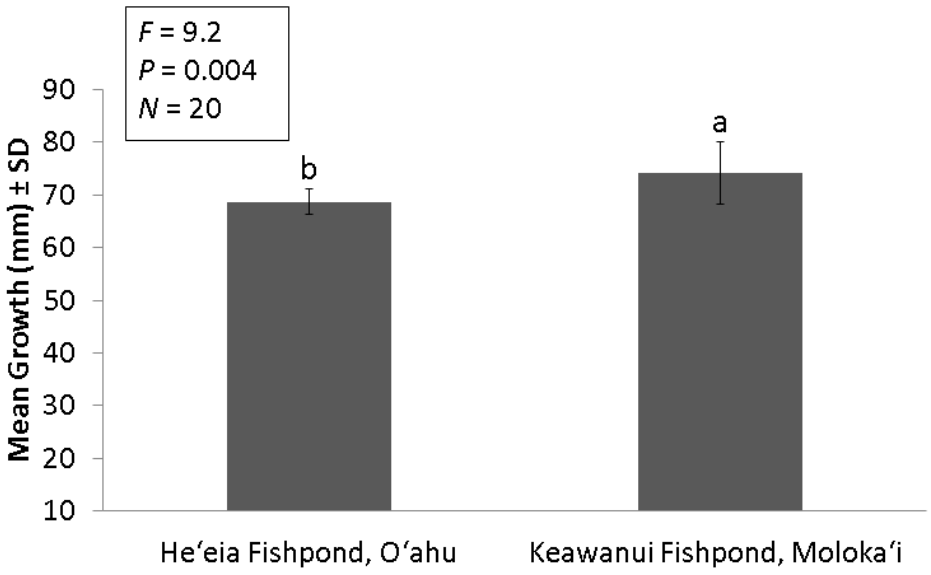

Figure 4. Mean shell length after 232 days of growth of all families of oysters in He'eia Fishpond, O'ahu, and Keawanui Fishpond, Moloka'i, HI, USA. Initial shell length was approximately 10 ± 5 mm. Means sharing the same letter designation are not significantly different. Results of a Two-sample T-test, α = 0.05.

Growth in He'eia Fishpond

There was no significant difference in growth between the five families of *C. gigas* raised at He'eia Fishpond, O'ahu ($P = 0.067$). All families of *C. gigas* displayed a steady increase in shell length during the 232 days following deployment (Fig. 5). Between the months of June and August there was a large decrease in mean shell length due to mortality of most oysters larger than 70 mm. The mortality resulted in a decrease in population to approximately 18% of the original number. The O'ahu oysters did not experience an extensive die-off in the months of July

and August, but during the month of September this group also suffered from high mortality. In the last 30 days of the He‘eia trial, the mean length of O‘ahu oysters declined from 80 mm to 70 mm while the other four families, EE, AE, AA, and Midori oysters, showed a rapid increase in mean shell length from 45 mm to 65 mm.

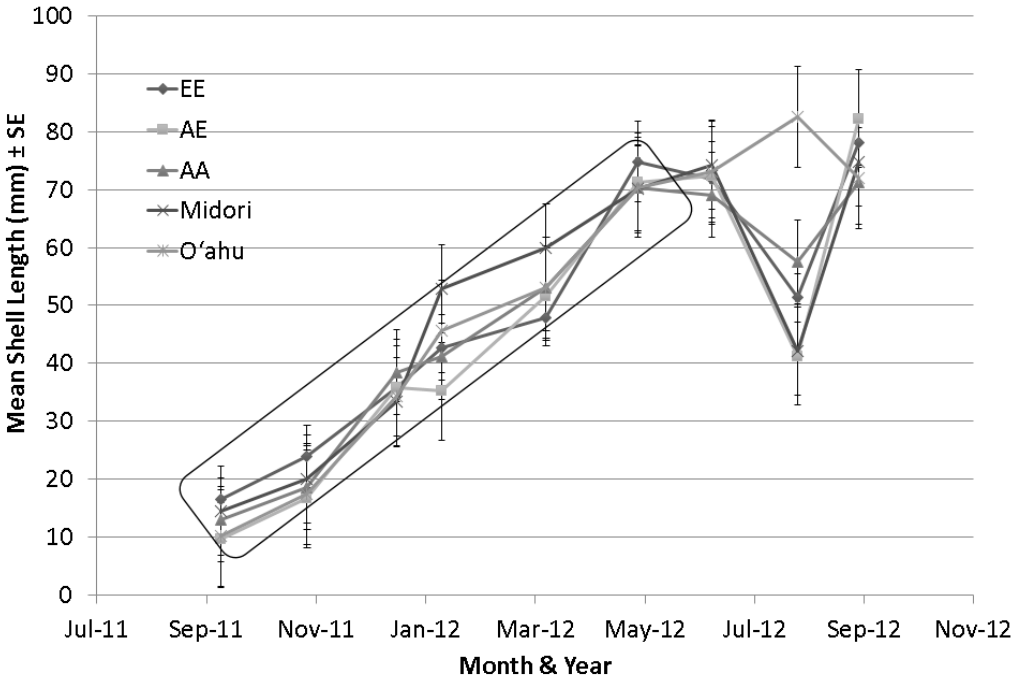

Figure 5. Mean shell length of *C. gigas* families EE, AE, AA, Midori and O‘ahu grown in He‘eia Fishpond, O‘ahu, HI, USA. The first 232 days of culture are shown in the circled region. Results of a Kruskal-Wallis test for mean shell length, $\alpha = 0.05$.

After 260 days of growth, the mean increase in shell length for all families was 58.6 ± 2.4 mm with a mean growth rate of 7.5 ± 2.1 mm per month. Family AE had the largest increase in median shell length of 61.8 ± 5.3 mm during the study, however the results were not significant (Fig. 6). Midori oysters had the lowest median shell growth of 56.1 ± 4.7 mm in He‘eia Fishpond.

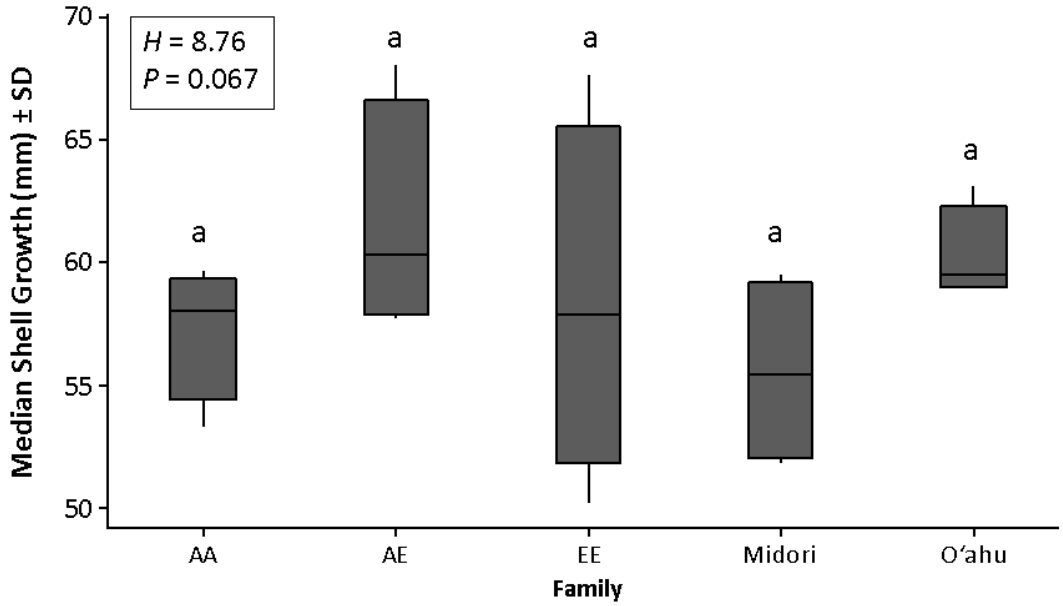

Figure 6. Change in median shell length of *C. gigas* families EE, AE, AA, Midori and O‘ahu during the first 232 days in He‘eia Fishpond, O‘ahu, HI, USA. Means sharing the same letter designation are not significantly different. Results of a Kruskal-Wallis Mood's Median test for increase in median shell length, $\alpha = 0.05$.

Growth in Keawanui Fishpond

In contrast to the He‘eia Fishpond study on O‘ahu, there was significant differences in growth between the five families grown in Keawanui Fishpond on Moloka‘i ($P = 0.035$). Shell length of all families in Keawanui increased steadily for the first 232 days with a mean growth rate of 8.7 ± 1.4 mm per month (Fig. 7). Oyster mortality was observed on all sample periods and across all families of oysters. The high-mortality phenomenon that affected larger oysters in He‘eia Fishpond in August happened concurrently in Keawanui. This mortality event further reduced the oyster population to just 5% of its original numbers.

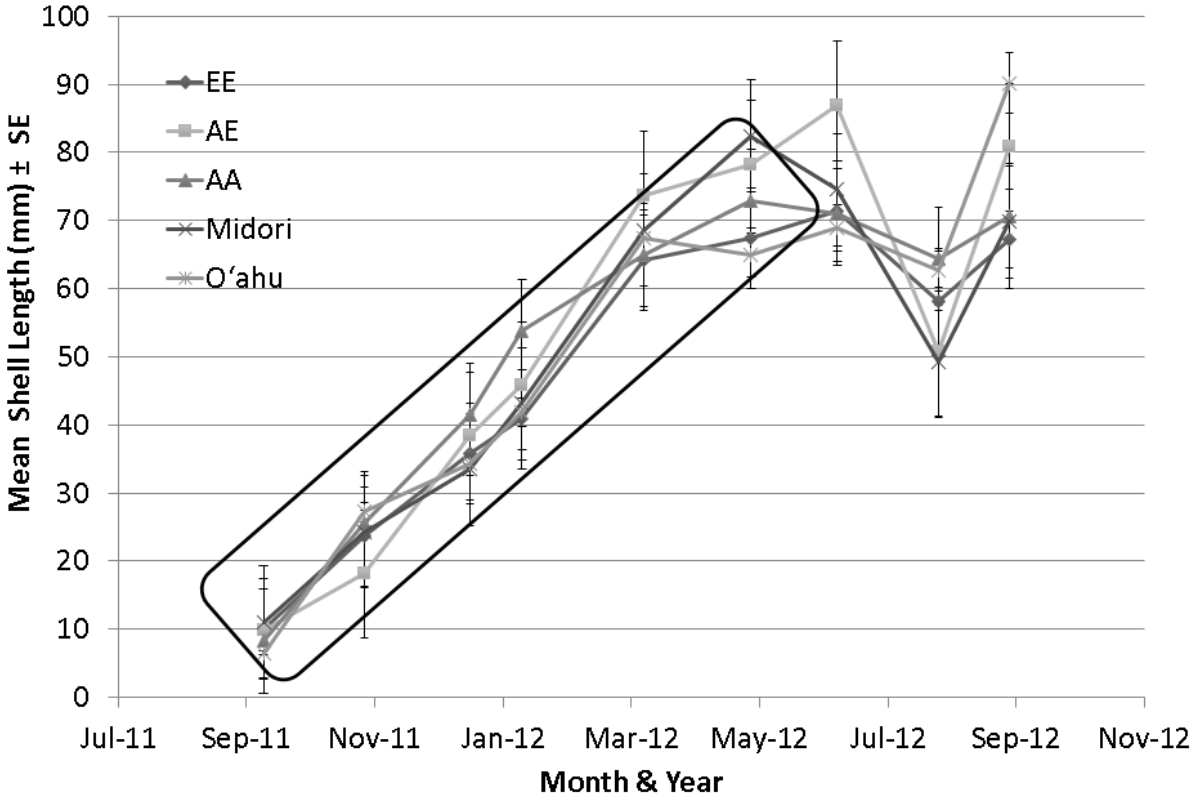

Figure 7. Shell length of *C. gigas* families EE, AE, AA, Midori and O'ahu grown in Keawanui Fishpond, Moloka'i, HI, USA. The first 232 days of culture are shown in the circled region. Results of a Kruskal-Wallis test for mean shell length, $\alpha = 0.05$.

The mean increase in shell length during the first 232 days for all families in Keawanui was 64.1 ± 5.9 mm (Fig. 8). Family AE had the highest median growth during the sampling period of 9.1 ± 1.3 mm per month. Families AA and Midori also had high growth rates of 8.3 ± 3.3 mm per month. All three families reached a in excess of 75 mm within 232 days of transplanting.

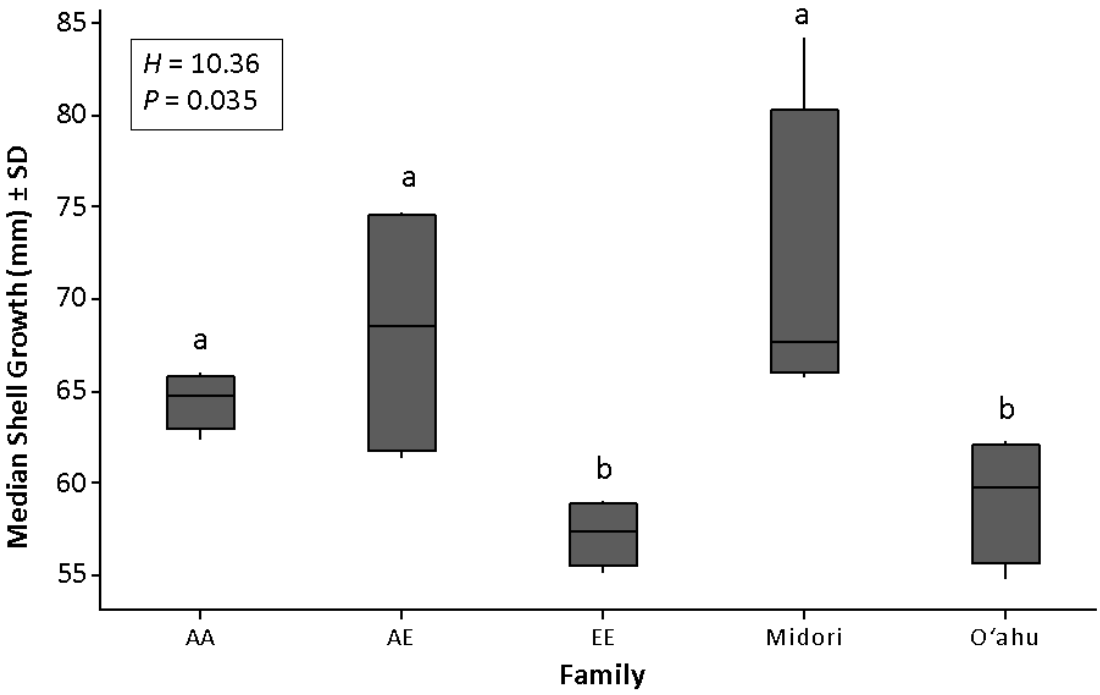

Figure 8. Change in median shell length of *C. gigas* families EE, AE, AA, Midori and O'ahu oysters during the first 232 days in Keawanui Fishpond, Moloka'i, HI, USA. Means sharing the same letter designation are not significantly different. Results of Kruskal-Wallis and Mood's Median test on increase in median shell length, α = 0.05.

Hilo Bay site characterization

Measurements of dissolved oxygen (DO), salinity, pH, and temperature at the study sites were found to be relatively consistent and within range of *C. gigas* tolerance (Table 1, Table 2).

Table 1. DO, salinity, pH, temperature, and chlorophyll *a* (± SE) at study site 1 and 2. Hilo Bay, Hilo, Hawai'i, USA.

Study Site	DO (mg/L)	Salinity (ppt)	pH	Temp. (°C)	Chl *a* (µg L^{-1})
1	6.06 ± 0.06	35.26 ± 0.15	8.02 ± 0.07	25.6 ± 0.85	0.32 ± 0.15
2	5.75 ± 0.64	34.70 ± 1.71	7.99 ± 0.07	25.9 ± 0.05	0.17 ± 0.06

Table 2. Tolerance range of *C. gigas* for DO, salinity, pH, and temperature (FAO, 2014).

DO (mg/L)	Salinity (ppt)	pH	Temp. (°C)
2.9 - 10.6	20 - 38	6 - 9.2	1.8 - 35

Hilo Bay oyster growth

Site 1

Our Hilo Bay growth data clearly shows a rapid increase in shell length for all varieties and at both sites immediately following the deployment of the oysters into the bay (Fig. 9, Fig 10). At Study Site 1, the median growth of triploid oysters was significantly higher than that of the MBP oysters ($P = 0.02$). Although MBP and triploid oysters started at 5 mm shell length, the growth rate of the triploid oysters started to exceed the diploid oysters by day 15 (Fig. 9). By day 30, the shell growth of both varieties started to decrease. The median shell length of the triploid oysters became significantly greater than that of the MBP oysters by day 43. The shell length of the triploid oysters continued to exceed the shell length of the MBP oysters for the duration of the study by up to 5 mm. Near the end of the experiment, the median shell length of the MBP oysters appeared to have reached a plateau at 30 mm while the triploid oysters continue to increase in length. After 115 days, the oyster cages at Site 1 broke off and drifted away, discontinuing data collection at Site 1.

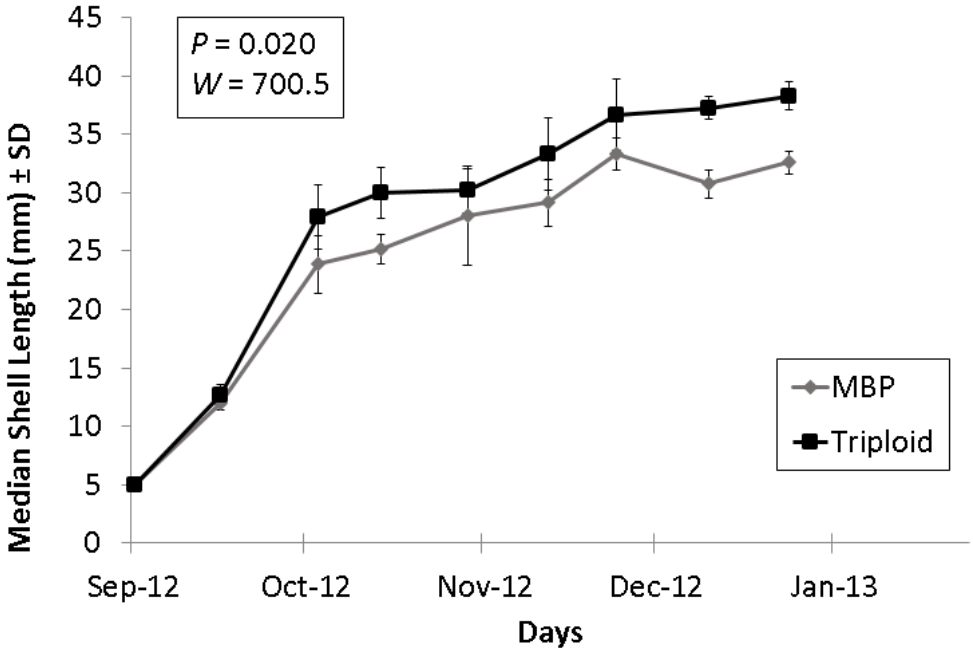

Figure 9. Median shell length of triploid and MBP *C. gigas* oysters grown in cages suspended at four m depth. Site 1, Hilo Bay, Hilo, Hawai'i, USA. Results from a Mann-Whitney test, $\alpha = 0.05$.

Site 2

To ensure a realistic commercial grow-out timeframe, the mean shell size after 300 days of oyster growth was analyzed (Fig. 10, circled area). The increase in shell length during the first 300 days of the study was also examined. Shell growth for all families was highly variable throughout the study.

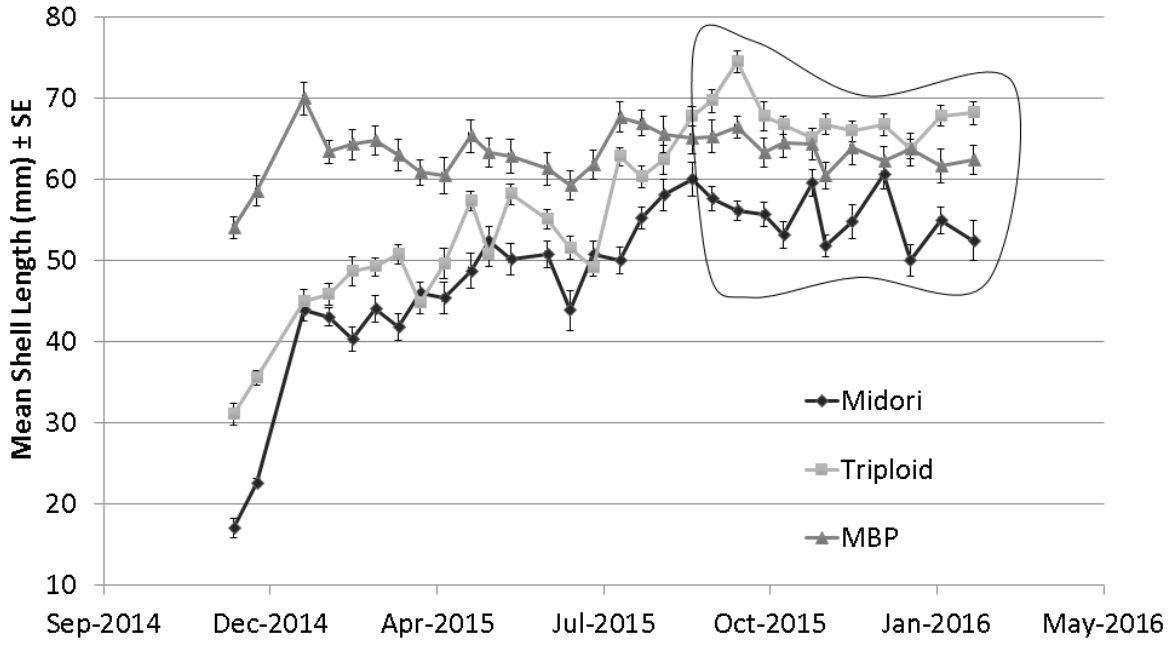

Figure 10. Growth of Midori, triploid, and MBP *C. gigas* oysters grown in cages suspended at four m depth. Site 2, Hilo Bay, Hilo, Hawai'i, USA. Mean maximum size shown in circled region.

Triploid oysters had the greatest increase in shell length and reached a significantly larger size than any other variety ($P < 0.0001$). Triploids were put into Hilo Bay at a starting size of 31 mm and grew at a mean rate of 3.7 ± 0.5 mm per month. Triploid oysters were significantly larger than other oyster varieties after 300 days with a mean shell length of 68.1 ± 4.1 mm, and continued to surpass other oyster varieties for the duration of the experiment (Fig. 10). The triploid oysters increased in mean shell length during most sample periods until the growth rate plateaued 300 days into the study.

The Midori oysters had an initial mean size of 15 ± 4.7 mm at the beginning of the study and a final mean size of 52.4 ± 6.2 mm after 300 days of growth. Midori oysters showed the fastest initial growth rate of 15.6 ± 7.9 mm per month for the first month before slowing to 3.2 ± 0.8 mm per month until day 300. The overall mean growth rate for the first 300 days was 3.5 ±

0.6 mm per month. After the first 300 days mean Midori shell length started to decline, most likely due to die-off of larger oysters. The MBP oysters were 54 mm in length at the start and ended with a mean length of 62.3 mm. The mean growth per month for MBP oysters was 0.8 mm per month, the lowest of all growth rates recorded.

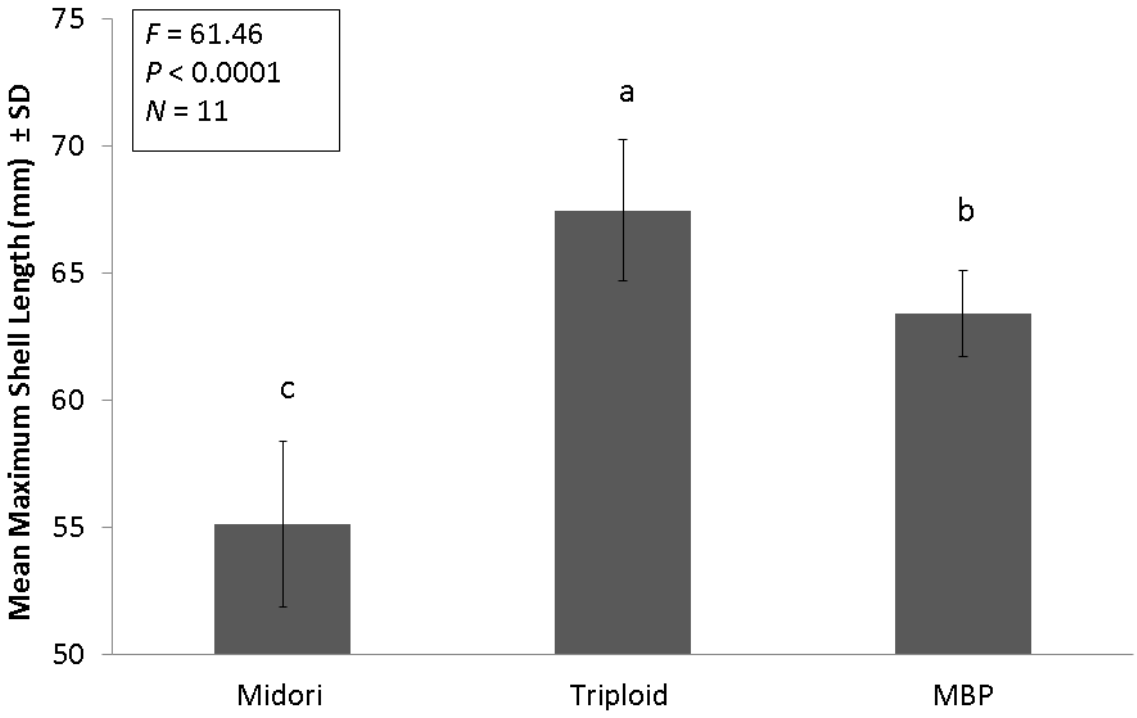

Figure 11. Mean maximum size of Midori, triploid, and MBP *C. gigas* oysters grown in cages suspended at four m depth. Site 2, Hilo Bay, Hilo, Hawai‘i, USA. Means sharing the same letter designation are not significantly different. Results of Kruskal-Wallis and Mood's Median test for mean maximum size, α = 0.05.

Condition index for He‘eia Fishpond oysters

Statistical analysis with an ANOVA test showed no significant effect of family on CI between the five families of *C. gigas* oysters grown in He‘eia Fishpond, (P = 0.08). Although the difference was not significant, Midori oysters had the highest mean CI 3.1± 0.7 of any family (Fig. 12).

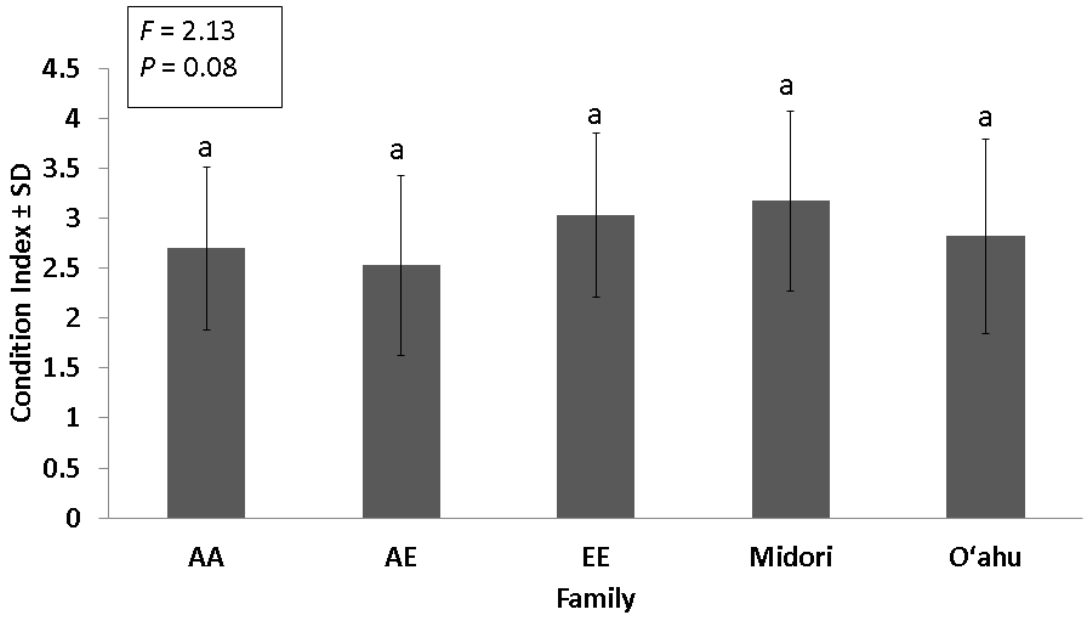

Figure 12. Condition index of families EE, AE, AA, Midori and O‘ahu *C. gigas* oysters grown in He‘eia Fishpond, O‘ahu, HI, USA. Means sharing the same letter designation are not significantly different. Results of ANOVA test and Tukey’s analysis on CI by family, α=0.05.

Condition index for Keawanui Fishpond oysters

Statistical analysis with an ANOVA test revealed a significant difference between CI of the five families at Keawanui Fishpond, (P = 0.01). Families AA and EE had a significantly higher CI than Midori or O‘ahu oysters (Fig. 13). Families AA and EE had a mean CI of 3.2 ± 0.8 and 3.1 ± 0.9 respectively. Family AE was not significantly different from any other of the four families. Midori oysters had the lowest CI values of 2.2 ± 0.9 followed by O‘ahu oysters with a CI of 2.4 ± 0.6. All families displayed relatively large standard deviations among samples as compared to He‘eia Fishpond.

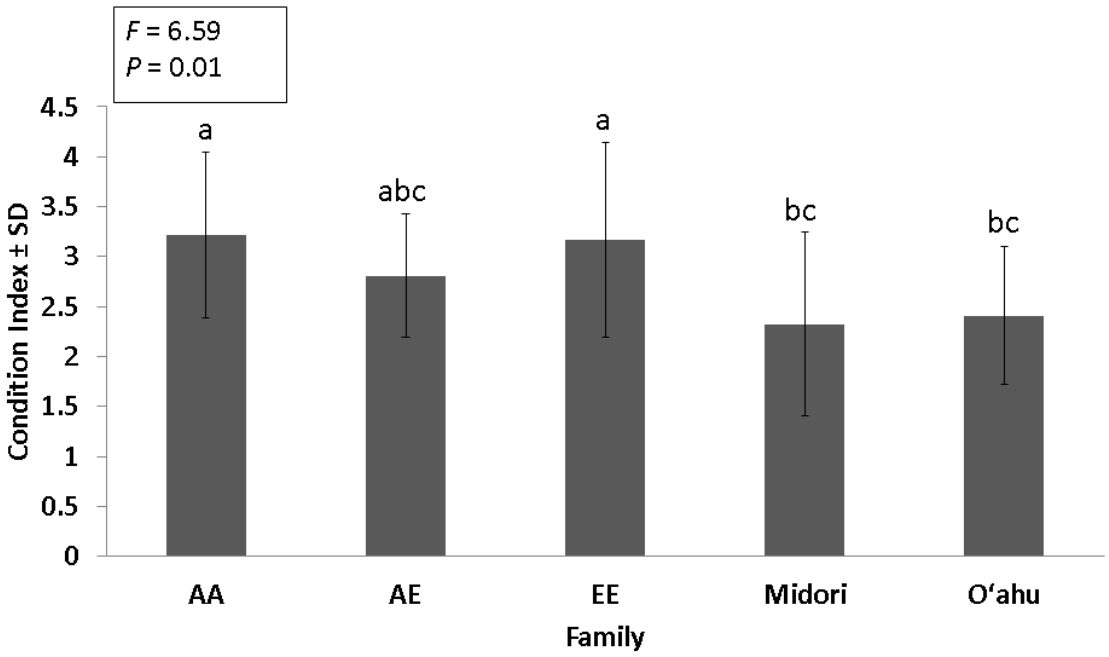

Figure 13. Condition index of EE, AE, AA, Midori and O'ahu *C. gigas* oysters grown in Keawanui Fishpond, Moloka'i, HI, USA. Means sharing the same letter designation are not significantly different. Results of ANOVA and Tukey's analysis, α = 0.05.

Condition index for Hilo Bay oysters

There was a significant difference between the CI of triploid, MBP and Midori oysters, (P = 0.002). Results of statistical analysis with a Kruskal-Wallis test show that the median CI of Midori oysters, at 7.0 ± 3.4, was significantly greater than that of MBP or triploid oysters (Fig. 14) The median CI for MBP and triploid oysters was 4.7 ± 2.0 and 4.6 ± 1.6 respectively.

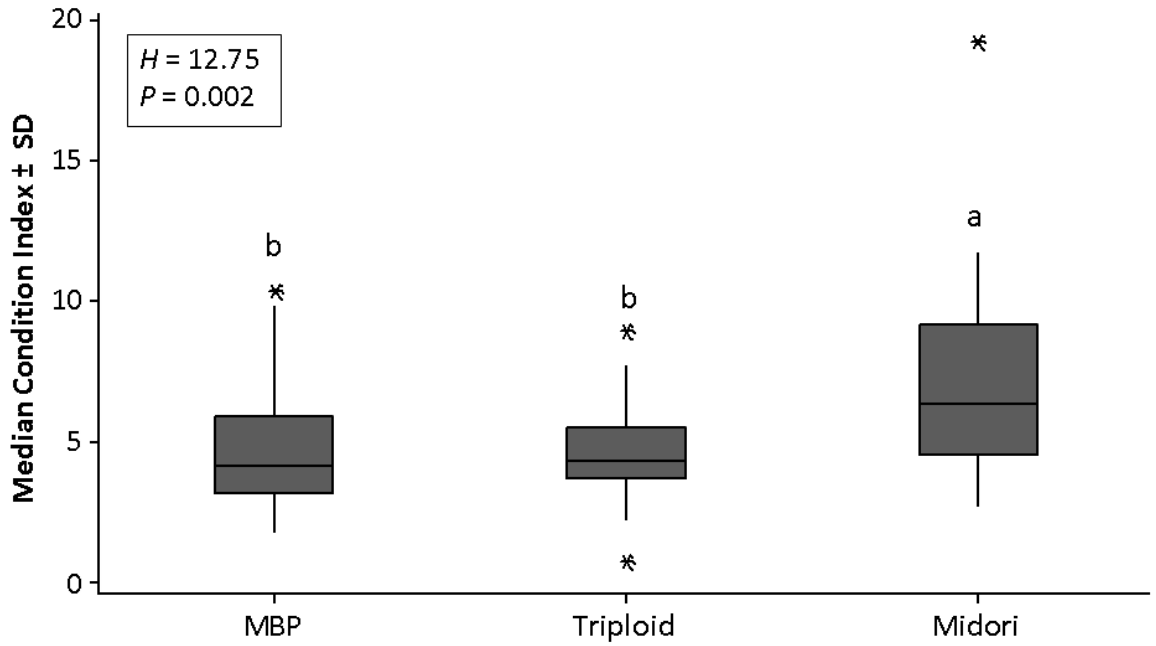

Figure 14. Condition index of MBP, triploid, and Midori *C. gigas* oysters from Hilo Bay, Hilo, Hawai'i, USA. Means sharing the same letter designation are not significantly different. Results of Kruskal-Wallis test and Mood's Median test, α = 0.05.

Growth compared to chlorophyll a

Chlorophyll *a* concentration (µg L^{-1}) has a significant effect on the variance of mean oyster growth ($P < 0.0001$). As hypothesized, chlorophyll *a* was positively correlated with oyster growth (Fig. 15). DO was not significantly correlated with oyster growth. The mean concentration of chlorophyll *a* and mean change in oyster shell length over a two-week period was calculated during a 115-day sampling period. About 40 % of the variance in oyster growth can be explained by the concentration of chlorophyll *a*. As chlorophyll *a* concentration increases, so does the biweekly mean of oyster growth. The highest mean concentration of chlorophyll *a* observed during a two-week period in October also correlates with the highest rate of oyster growth recorded. During this time, with a mean chlorophyll *a* concentration of 5 µg L^{-1}, oysters

grew 13.6 ± 2.9 mm. On the other end of the spectrum, during the periods of low chlorophyll *a* concentration with mean values below 1.2 µg L^{-1}, the oysters grew only 2-3 mm over a two-week span.

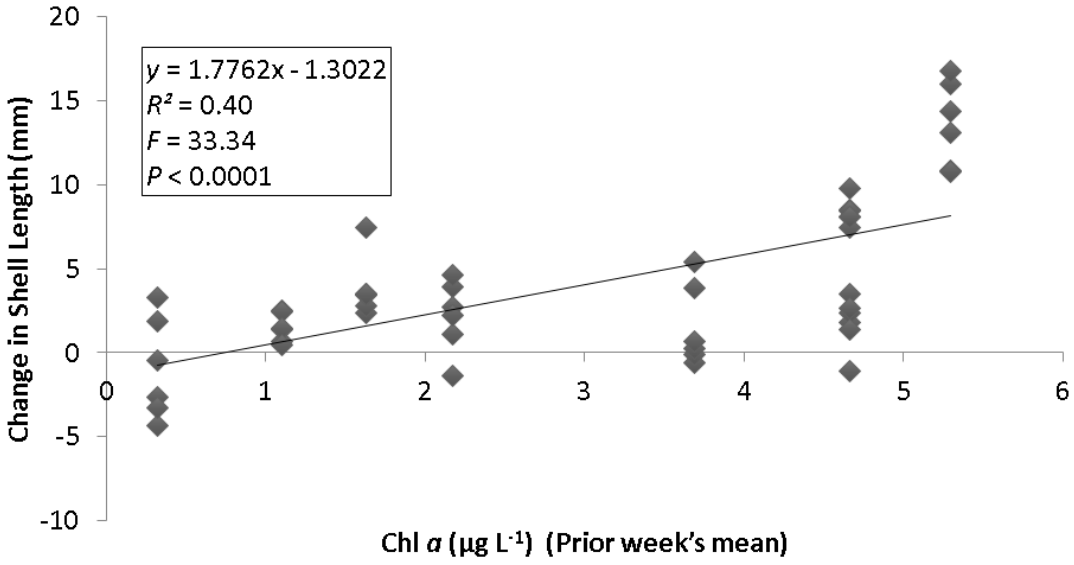

Figure 15. Mean change in shell length vs. mean concentration of chlorophyll *a* during two-week time spans within a 115-day sampling period. Site 1, Hilo Bay, Hilo, Hawai'i, USA, α = 0.05.

Predation in Hilo Bay

The number of predators found in each cage has a significant influence on the variance in oyster mortality, ($P < 0.0001$). Approximately 57 % of the variance in oyster mortality observed can be attributed to predation (Fig. 16). Mortality was higher (45%) among Midori oysters than MBP or triploid oysters (Table 3). Midori oysters also had shells with much lower dry weights than triploid or MBP oysters. Triploid and MBP oysters had a mean mortality of 19% and 34%, respectively.

Table 3. Total percent mortality and dry shell weight of each variety cultured in Hilo Bay during a 431-day study. Hilo, Hawai‘i, USA.

Variety	Midori	Triploid	MBP
% Mort.	45	19	34
Shell wt. (g)	6.6 ± 3.1	16.6 ± 6.2	19.7 ± 6.3

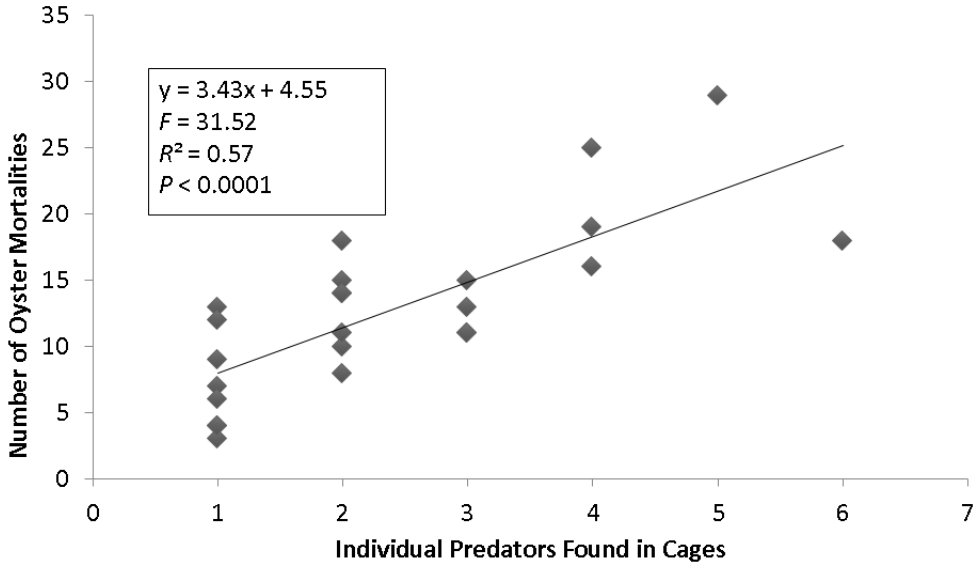

Figure 16. Regression analysis for presence of predators vs. mortality of *C. gigas* oysters grown in Hilo Bay, Hilo, Hawai‘i, USA.

Treatments for <u>Polydora websteri</u>

The SSSP treatment was significantly more effective than any other treatment at removing *P. websteri* (Fig. 17, $P < 0.0001$). The SSSP treatment resulted in the highest level of *P. websteri* mortality and removal from oyster shells. Oyster mortality was no higher with SSSP than with the control (Fig. 18). This treatment also resulted in nearly double the *P. websteri* removal as the next most effective removal treatment. The SSSP treatments removed approximately 95% of all *P. websteri* from the infected oysters. Most *P. websteri* died shortly after exiting the shell but about 15% were still alive 24 hours post treatment (Fig. 19). Treatment ABD was about 50% effective at removing *P. websteri* but also caused mortality in about 36% of

the treated oysters. Treatment AD caused the most oyster mortality at approximately 45% and was only effective at removing about 30% of *P. websteri* in samples. Treatments BD removed about 21% *P. websteri* and resulted in relatively low oyster mortality of less than 6% in treated oysters. Treatment FD resulted in very little oyster mortality but also low *P. websteri* removal.

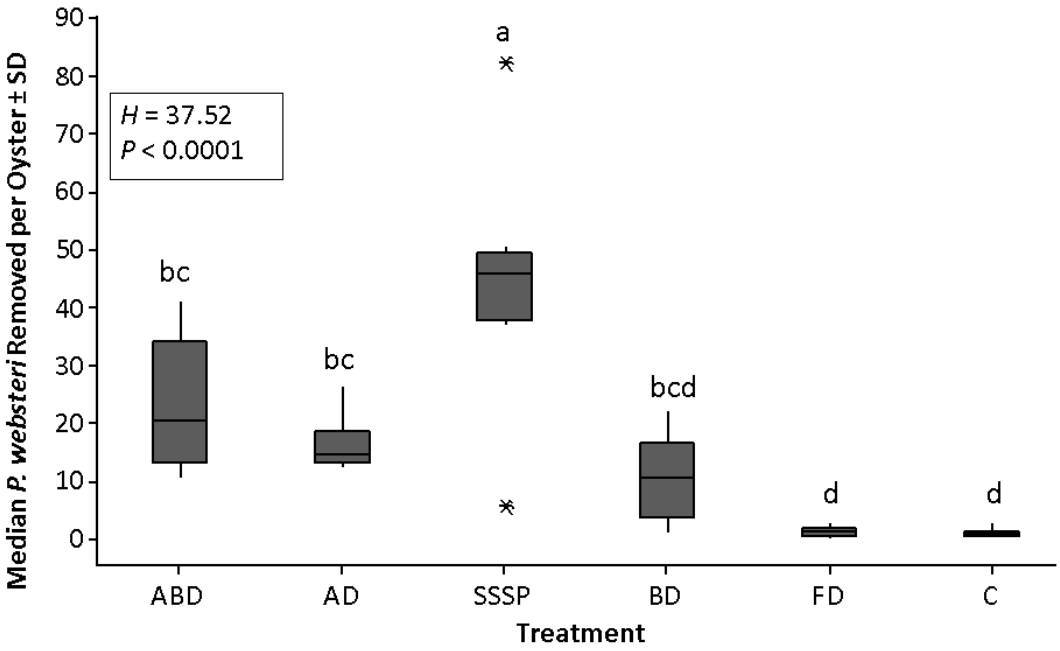

Figure 17. Median *P. websteri* counted after exiting infected oysters, forty-eight hours post-treatment. Means sharing the same letter designation are not significantly different. Results of Kruskal-Wallis and Mood's Median test on total *P. websteri*, α = 0.05.

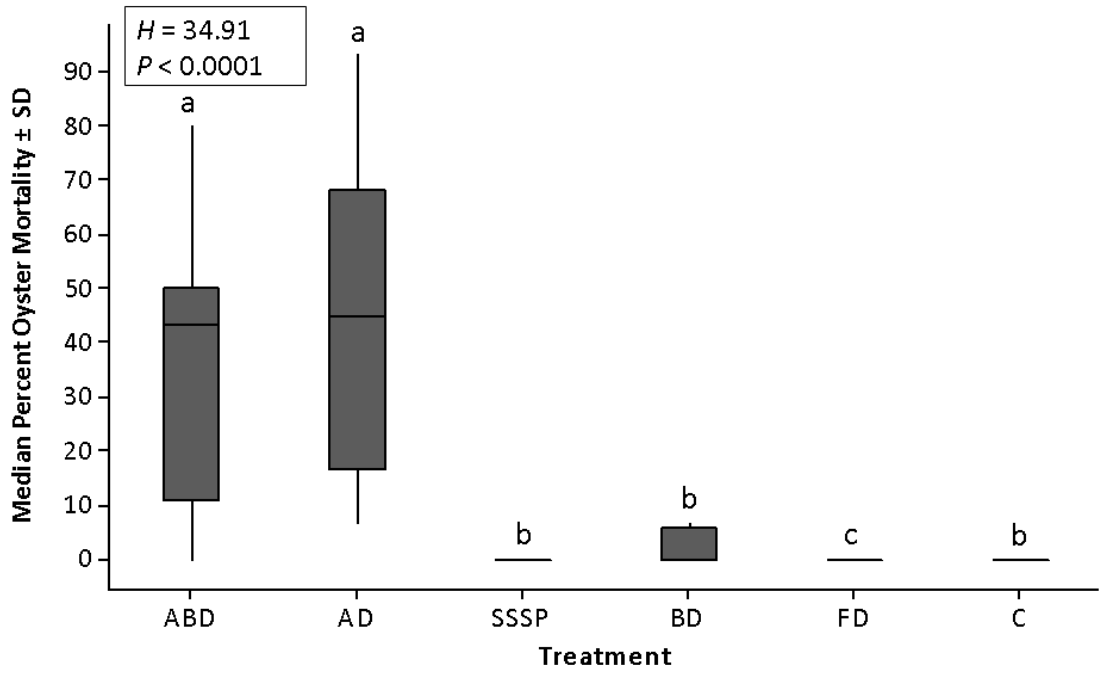

Figure 18. Average percent oyster mortality associated with each treatment, forty-eight hours post-treatment. Means sharing the same letter designation are not significantly different. Results of Kruskal-Wallis and Mood's Median test on total oyster mortality by treatment, α = 0.05.

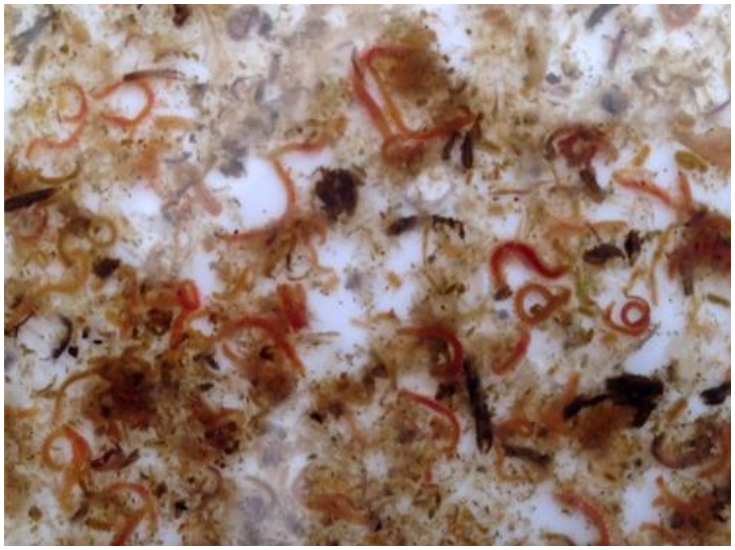

Figure 19. Photograph of *P. websteri* after exiting oysters twenty-four hours after treatment.

Discussion

Oyster growth in fishponds

The significantly faster growth rates observed in Keawanui Fishpond may indicate better overall growing conditions than those found at Heʻeia Fishpond. These results build on the work of Sparks (1963), whose assessment concluded that the most suitable oyster grow-out locations in the Hawaiian Islands were located on Molokaʻi. In addition to Keawanui Fishpond, there are numerous other fishponds along Molokaʻi's southeastern shore with a high degree of potential for oyster culture (Sparks (1963). Research will need to be performed to confirm that these fishponds can achieve similar growth rates to those seen at Keawanui.

Family AE from the MBP had the highest median growth rate in both fishponds. Family AE also had less variation in size and growth rate compared to other families making it particularly attractive for oyster cultivation because of its predictable growth. The large decrease in mean shell length of all families near the end of the fishpond study was likely due to the mortality event that afflicted larger oysters in both fishponds. The subsequent increase in mean shell length during the last month of the study may be explained by the removal of larger dead oysters from the cages, which may have provided the smaller oysters with increased access to food resources, resulting in rapid growth.

Similar studies on the growth of *C. gigas*, were conducted by Brick (1970) in Kahaluʻu Pond, Oʻahu. Brick's raft culture method suspended oysters in the water column and was functionally similar to the long-line cage suspension used in this study, allowing us to make comparisons. Results from Brick (1970) show a wide range of growth rates, ranging from 2.8 mm per month to 5.5 mm per month, with a mean of 4.5 mm per month. The mean growth rate at Keawanui was 1.2 mm per month faster than at Heʻeia during the first 232 days and was nearly

double the growth rates seen from Brick (1970). The shell growth rate documented in this study is higher than the shell growth rates published in many previous studies conducted around the world (Table 4).

Table 4. Location, mean growth, and literature reference for growth studies.

Location	Mean Growth (mm per month) ± SD	Reference
He‘eia, O‘ahu, HI, USA	7.5 ± 2.7	This study
Keawanui, Moloka‘i, HI, USA	8.7 ± 1.4	This study
Hilo, Hawai‘i, Site 1, HI, USA	7.5 ± 2.1	This study
Hilo, Hawai‘i, Site 2, HI, USA	3.0 ± 1.7	This study
Kahalu‘u Pond, O‘ahu, HI, USA	4.5 ± 1.8	Brick 1970
Laguna La Cruz, Northwest Mexico	8.2 ± 2.3	Chávez-Villalba et al. 2010
North Sea, Germany	5.1 ± 1.8	Pogoda et al. 2011
Gamakman Bay, South Coast Korea	6.4 ± 0.8	Mondol et al. 2016
Vancouver, Canada	3.7 ± 0.7	Brown 1988

Research carried out by Pogoda et al. (2011) recorded a mean growth rate of 5.1 ± 1.8 mm per month in Germany, well below the mean growth rates seen in the fishpond study. Similar growth rates to those seen in this study have been found by Chávez-Villalba et al. (2010) during a growth experiment done in northern Mexico. They reported shell growth of about 20 mm during the first month, similar to the Midori strain cultured in Hilo Bay and early growth rates from Keawanui Fishpond. They further noted that increased stocking density resulted in decreases in growth and final size. Comparable to this study, they witnessed the most growth during the first 7 to 8 months following oyster deployment. Overall, they documented growth rates of about 9 mm per month when cultured in a protected lagoon and about 6 mm per month when cultured outside the lagoon in the Gulf of California. These results are very similar to the differences found between the fishponds and Hilo Bay. In this study, consistently higher growth rates were documented in the fishponds than in the comparatively clear waters of Hilo Bay. The

oysters grown by Chávez-Villalba et al. (2010) reached marketable size in one year after deployment, a timescale similar to the fishpond trials.

Oyster growth in Hilo Bay

Triploids performed better than the other varieties in terms of growth and survival. The triploid oysters grew to the largest size, and had the highest mean growth rate of any variety at both study sites in Hilo Bay. The differences in growth rates between triploids and the other varieties grown in Hilo Bay supports previous studies that have found that triploids exhibit increased growth of up to 40% compared to diploids (Guo & Allen 1994a). Triploids may also survive in a much wider range of climates and conditions than diploids of the same species (Nell 2002, Guo & Allen 1994a). Research also shows that as environmental conditions for *C. gigas* growth improve, the growth difference between diploid and triploid oysters also increases significantly (Nell 2002).

The fast initial increase in shell length followed by slowing growth that occurred at the Hilo Bay sites is common in growth studies (Chávez-Villalba et al. 2010). Brick (1970) documented faster growth rates with smaller oysters and noted a slowing rate as the oysters matured past one year. Pogoda et al. (2011) showed that oysters devote most of their resources to shell growth in their first year of life and reallocate the bulk of their energy towards soft tissue production and glycogen in later years.

At Hilo Bay Site 2, shell length peaked at 55 to 65 mm in length for all varieties after 300 days. Thereafter, mean shell length stopped increasing and became highly variable without increasing for the next 4 months (circled area, Figure 10). In contrast to the Hilo Bay data, similar studies that deployed oysters at a greater 30 mm initial size, documented nearly continuous growth up to harvest at 80-90 mm. Furthermore, Brown (1988) recorded continuous

growth eventually reaching 90-100 mm in length in ten months for oysters deployed at over 45 mm. Mondol et al. (2016) reported linear growth rates of 6.4 ± 0.8 mm per month for *C. gigas* oysters deployed at over 30 mm in size in Gamakman Bay, Korea and never reached a plateau before harvest at 360 days. Bishop & Hooper (2005) found that shell growth in oysters may stagnate because of low phytoplankton availability or other environmental stressors. Overall, if the findings reflect typical conditions at Hilo Bay Site 2, this site may not be suitable for oyster culture.

Condition index for fishpond oysters

Similar research by Brick (1970), found that the CI rarely approached commercial quality after analyzing *C. gigas* oysters grown in Kahaluʻu Pond, Oʻahu. Studies on condition index show a wide range of 3.4 to 11.8, with acceptable commercial values of 5 and higher (Pogoda et al. 2011). This study shows the highest CI in the Midori and EE families with a mean CI value of just over three, well below the commercially acceptable level. This may be in part due to an error in experimental design, which resulted in confounding factors making conclusions difficult. The oysters for both fishponds were purged in clean water for five days directly preceding CI analysis. The purging may have resulted in reduced body weight of the oyster in relation to shell weight, skewing the analysis and leading to a low CI. Another possible confounding factor may have been that the oysters used for the CI had recently spawned thus lowering the expected CI by half.

Condition index for Hilo Bay oysters

Although the CI of the Midori oysters was an acceptable level, the inherently thinner shells in the oyster variety indicate that the results must be interpreted cautiously. Comparatively little is known about Midori oysters compared to MBP or triploids. The Midori strain which was

used in this study originated near the Midori River in southern Japan (Langdon, pers. comm. 2016) and may have a higher tolerance to warmer environments than most *C. gigas.* An additional characteristic for Midori oysters that was noted in this study was thinner shells compared to the triploid and MBP oysters. Due to the environmental conditions of Hilo Bay, it was originally thought that only triploids may build enough glycogen to be of sufficient quality for commercial cultivation. However, it was the Midori strain that proved to have the highest average CI of 7.0 ± 3.4. The Midori oysters grown in this study show a wide range of CI values. Considering that the CI analysis uses a ratio of dry meat weight to shell weight, it is possible that the high CI value observed in the Midori oysters was partly due to their thin, not their ability to "fatten" in warmer waters. This essentially means that the results of the Hilo Bay CI analysis should be interpreted with caution due to slight morphological differences in shell weight between oyster varieties (Table 3). Based on the numbers obtained from the CI analysis, lack of food and environmental conditions may hinder some varieties of oysters from reaching sufficient table quality for commercial cultivation at study Site 2. Some studies have reported seasonal condition index changes with peak condition indexes found between March and July and the lowest values found in November to February (Bayne 2009). The CI of oysters grown in Hilo Bay was only measured in February, and may not be representative of the average CI year-round. Due the confounding factors in the CI study of both the fishpond and Hilo Bay, no clear conclusions can be drawn. The benchmark of a CI of 5 is considered essential for the highly-valued half shell market. Future research should include comparative CI analysis from multiple fishponds and sites in Hilo Bay during different times of the year.

Chlorophyll a as a predictor for growth

Our findings match prior studies that found higher chlorophyll levels lead to increased soft tissue production and growth (Grangeré et al. 2009). Research by Cassis et al. (2011) in British Columbia also found that oyster growth was strongly correlated with phytoplankton abundance, particularly diatoms. Research by Brown and Hartwick (1988) analyzed the effects of chlorophyll *a* and other parameters on oyster growth near Vancouver, British Columbia. They reported the greatest increase in *C. gigas* shell growth at sites with the highest concentrations of phytoplankton if there were biologically appropriate salinity and temperature levels. They further documented that study sites with lower concentrations of phytoplankton result in oysters with reduced soft tissue mass to shell ratios. Suggesting that in sub-optimum food conditions, oysters may partition more energy resources towards shell growth than soft tissue production. In the analysis of the chlorophyll levels near Hilo Bay Site 1, there were a few periods where chlorophyll *a* was relatively high at 3.7 µg L^{-1} but oyster shell growth was relative low, 0-3 mm per month. In the Vancouver study, they came across similar results and suggested that the reduced shell growth occurred from adverse water quality parameters that coincided with the relatively high levels of phytoplankton. Large storm events over the Hilo Bay watershed have been shown to cause fluctuations water quality parameters including temperature, salinity, and chlorophyll *a* concentration, factors known to effect oyster growth (Weigner & Mead 2009).

Unlike the seasonal phytoplankton blooms documented by previous studies (Cassis et al. 2011, Brown & Hartwick 1988, Mondol et al. 2016, Goulletquer et al. 1998), studies of Hilo Bay show that primary productivity is largely influenced by precipitation in the Hilo watershed (Weigner & Mead 2009). Weigner et al. (2010) found that the flow from Wailuku River produced during severe rainstorms, can replace the entire water column in Hilo Bay in under 15

days. Further investigations found that primary productivity from phytoplankton, and respiration from zooplankton and other organisms decreased to the lowest recorded values after high flow conditions form the Wailuku River (Mead et al. 2010). Chlorophyll *a* levels were reduced 93% compared to concentrations during low-flow conditions (Weigner & Mead 2009). This large influx of fresh water into Hilo Bay led to drastic decreases of chlorophyll *a*, probably because primary production was hampered by salinity fluctuations and being severely diluted. The ensuing fluctuations in available phytoplankton may have been a contributing factor to the fluctuations in growth documented in this study.

Research has shown that during low river flow conditions in Hilo water shed, surface sea waters are autotrophic approximately 74% of the time (Mead et al. 2010). Indicating primary productivity was highest in Hilo Bay during low flow conditions common during the summer (Weigner & Mead 2009, Mead et al. 2010). During these prevailing low flow conditions, gross primary productivity was found to be up to 3.3 times respiration values. This indicates that surface water metabolism is mostly influenced by abiotic processes under conditions of high flow perturbations and biotic factors during low flow conditions (Mead et al. 2010). Weigner & Mead (2009) reported that the highest levels of chlorophyll *a* were found near the center of the bay.

The more rapid growth seen in the fishponds in comparison to Hilo Bay may have been due to low phytoplankton availability at the culture site. Reports on water quality analysis of Hawaiian fishponds indicates that the mean chlorophyll *a* concentration of about 6 $\mu g\ L^{-1}$, is well above the mean of found in Hilo Bay at Site 1 and Site 2 of $0.32 \pm 0.15\ \mu g\ L^{-1}$ and $0.17 \pm 0.06\ \mu g\ L^{-1}$, respectively (EPA Report 2003). These concentrations are also much lower than the mean concentration of $3.6\ \mu g\ L^{-1}$ documented by Mondol et al. (2016) in Gamakman Bay, Korea.

Chlorophyll *a* concentrations were higher near the surface of Hilo Bay than at the 4 m culture depth so it is probable that the oysters grown in Hilo Bay may have had faster growth rates if they were cultured near the surface, where phytoplankton is at its highest densities. This suggests that reduced food availability may have been the most significant factor in the reduced growth seen in Hilo Bay compared to the fishponds.

Future research analyzing phytoplankton composition and concentration should be carried out in fishponds and other potential grow-out sites. This is necessary to establish if oyster growth is similarly correlated with phytoplankton abundance in other growing locations. Research from this study indicates that chlorophyll *a* is perhaps the best predictor for growth and should be analyzed when finding a suitable location to conduct grow-out operations in Hawaiian waters.

Removal of Polydora websteri

The results of the *P. websteri* eradication treatments clearly show the effectiveness of SSSP treatment above all other treatments in regards to both oyster mortality and *P. websteri* removal effectiveness. Oysters treated with the SSSP appeared to have better health and increased shell growth in the following months compared to other treatments but measurements were never taken to confirm this. These benefits may have been due to the effectiveness of the SSSP, and the lack of harmful *P. wevsteri* in the oysters. The level of *P. websteri* infection in most samples was 40-60 worms per oyster. These infection levels were similar to Sato-Okoshi et al. (2013) who counted 30-80 worms per infected oyster during a study in Eastern China.

Treatments such as BD, a hyper saline dip followed by air drying adapted from Bishop and Hooper (2005) were relatively ineffective at removing *P. websteri*. Bailey-Brock and Ringwood (1982) reported marginal success with hypersaline treatments, and like the 6%

mortality in this study, further noted that only healthy oysters with undamaged shells could withstand the hours of treatment required to kill *P. websteri* living in their burrows. Bishop and Hooper (2005) reported significant reductions in *P. websteri* from infection levels of approximately 40 % down to only 5% with treated oysters using hyper-saline dips followed by air drying. They noted no reductions in growth because of the treatment. Their study did record about 5% greater mortality than untreated oysters because of the stressful treatment. They further emphasized that the increase in the number of marketable oysters because of the treatment would likely offset the cost of the treatment itself (Bishop & Hooper 2005).

Treatments ABD and AD resulted in removal of *P. webster*i but also caused about 35-45% oyster mortality in samples making these treatments undesirable. Not unlike the experiments with treatment (FD), Bailey-Brock and Ringwood (1982) found that fresh water and drying treatment ineffective unless dip and drying times were extended to six hours. Although *P. websteri* removal rates were relatively low, this treatment is attractive because of the relatively cheap cost of rinsing and soaking oysters in fresh water and allowing them to air dry. All other methods require additions of hot water, ice, salt, or some other such treatment.

The effectiveness of the SSSP treatment is only effective and safe for oysters if preformed correctly. If oysters are kept too long in the icy brine, their internal tissue also freezes resulting in mortality (Cox et al. 2012). Along the same lines, if the oysters are kept dry for too long following treatment they also suffer high mortality rates. The SSSP treatment is highly effective at removing *P. websteri*, and the treatment has also been shown to be effective at killing epibionts including barnacles, flatworms, and oyster overset, with no mortality if exposure time is limited to 75 seconds (Cox et al. in 2012). All of these are documented problems associated with oyster culture in Hawaiian waters (Bailey-Brock & Ringwood 1982, Brick1970, McCarty,

pers. comm. 2016, Sparks 1963). Although performing the SSSP treatment on oysters in the field may prove difficult, many culture systems such as cages suspended from long lines and floating raft culture systems can be transported to a treatment facility near shore where they could be treated for biofouling and pests. Ice and salt are relatively cheap in Hawai‘i and could be used in combination to make large scale SSSP treatments.

Royer et al. (2006) documented severely reduced growth and meat quality as well as meat and shell weights in oysters infected with *P. websteri.* McCarty also reported continuous re-infection when they temporarily suspended oyster grow out operations at Kualoa Ranch due to heavy infestations of *P. websteri*. Some research (Royer et al. 2006, Handley & Bergquist 1997) suggests seasonality associated with *P. websteri* infections due to their typically summer reproductive cycle and recommends moving oyster stocks to deeper waters less prone to *P. websteri* infection during this time. Due to Hawai‘i’s relatively warm water, 21-25°C, and high food levels, *P. websteri* is in a continuous reproduction cycle, making eradication of *P. websteri* in Hawai‘i especially difficult because oysters are constantly being re-infected with new larvae (Bailey-Brock & Ringwood 1982).

P. websteri is a concern in Mahurangi Harbour, northern New Zealand where researchers Handley & Bergquist (1997) experimented with fortnightly washing and passive intertidal exposure as a means of prevention. They determined that if oysters were positioned just above extreme low water neap levels and at least 0.5 m above the bottom the *P. websteri* infections would be minimized if not completely avoided. Along the same lines, Royer et al. (2006) in Normandy, France recorded significantly reduced *P. websteri* infestation levels in *C. gigas* oysters reared at the intertidal zone. More precisely they found strong negative correlations between *P. websteri* and intertidal exposure of host oysters. Analysis of condition index of

oysters regularly exposed to air during low tide by Mercado-Silva (2005) found no significant difference with oysters grown in deeper waters that were never exposed to air, indicating no alterations to meat quality from exposure. In restored Hawaiian fishponds, the makaha (sluice gates) could be used to manipulate the water levels in the pond to induce the oysters to periodic drying. Future research should experiment with tidal induce drying by using long-line systems that facilitate brief intertidal exposure.

Oyster mortality in fishponds

Much like a previous study by Bailey-Brock and Ringwood (1982), this study also found evidence of predation by crabs, *Stylochus* flatworms, and *Cymatium* snails. Substantial algae and encrusting sponge growth was observed on cages and oysters during all sampling periods and only increased as the experiment continued. There were two species of encrusting sponge that appeared to suffocate the oysters by completely encapsulating them. The large mortality rates observed in the fishpond trials are congruent with findings from previous studies by Brick (1970) in Kaneohe Bay. Brick (1970) also found that mortality events were preceded by periods of little to no shell growth, a phenomenon noted in this study before the mass mortality events in July - August. Although restored fishponds have one or more makaha, water quality management and temperature control, two of the most important considerations for growing oysters, is difficult.

Oyster farm manager, Kuʻuipo McCarty reported seeing high rates of mortality in Moliʻi fishpond annually during the months of August and September. During this time, the pond reaches thermal maximums more than 30 °C, and undergoes phytoplankton blooms and subsequent crashes followed by oxygen minimums. Goulletquer et al. (1998) found that "summer mortality" has been described worldwide and is usually correlated with the culmination of a variety of stressors. These stressors include high temperatures, seasonal spawning,

phytoplankton blooms, and opportunistic pathogens. Summer mortality events can be devastating sometimes leading to 60 to 90% mortality in oyster stocks (Mondol et al. 2016). Mondol et al. (2016) also concluded mass mortalities in Korea were due to a combination of temperature fluctuations and stresses following natural spawning events. This is partly because the conditions that lead to natural spawning events such as warm waters and high phytoplankton availability, can also lead to increased risk of pathogens and disease for the already stressed oyster (Mondol et al. 2016). Goulletquer et al. (1998) concluded that nutrient loading and anthropogenic influences exacerbate increasing temperatures and large phytoplankton blooms which in turn can lead to depletion of oxygen in the water column and sexual maturation in viable oysters. This subsequently, causes severe physiological stress on the oyster often forcing them to spawn and leaving them physiologically weakened and susceptible to pathogens (Goulletquer et al. 1998). Analysis of summer mortality in the Irish Sea by Malham et al. (2009) documented that mortality was highest in July to August and coincided with nutrient loading and thermal maximums which in turn led to eutrophication of the waters. Many of these factors likely contributed to the mass mortality event recorded in both fishponds.

The timing of the large die-off event in the fishpond trials matches with the months of known summer mortality in fishponds, spawning season, and conditions conducive to high summer mortality. Utilizing sea surface temperature data taken in Kaneohe Bay, O‘ahu from the Global Ocean Acidification Observing Network, it was found that the large mortality event in both fishponds occurred in August during the hottest months of the year. This coincided with the addition of small spat 1-4 mm in length covering cages and available oyster shells, suggesting that the stress of spawning may have contributed to high levels of mortality. Research by Sparks (1963) recorded that naturalized populations of *Crassostrea virginica* in West Loch, Pearl

Harbor spawn naturally during the late summer near the time period recorded in this study. Future studies should investigate potential summer mortality mitigation methods such as relocating oysters to more stable waters temporarily.

Mortality not associated with the summer mortality event documented in this study was likely due to a variety of predators and fouling by epibionts. Oysters and cages in fishponds became heavily fouled with encrusting sponges, algae and other epibionts reducing water flow through the cages and perhaps contributing to mortality rates. Crabs and other predators were often found inside the cages when they were being tended, likely contributing to the mortality observed.

Oyster mortality in Hilo Bay

The number predators found in oyster cages in this study have been shown to account for 57.0 % of the variance in oyster mortality. The highest predation rates in Hilo Bay were in the Midori oyster cage. This may have been because the Midori oysters had thinner shells, perhaps making them more susceptible to predation from crabs capable of breaking thin oyster shell with their claws. Predatory crabs tentatively identified as *Thalamita integra* and *Callinectes sapidus,* were found inside the oyster cages, and are known to be capable of breaking oyster shells with their claws. Compared to Midori oysters, the MBP and triploid oysters had much thicker shells which most likely contributed to the tenfold increase in survival seen in this study. Furthermore, the MBP and 3N oysters were deployed at considerably greater average size than the Midori oysters which may have further reduced their potential for predation compared to Midori oysters. Many of the Midori shells had large wedge shaped pieces missing from the front of the oyster where the shell is the thinnest, most likely due to crab predation. Oysters less than a year old are especially susceptible to this form of predation (Sparks 1963). These results illustrate the

importance of weekly cleaning and removal of predators from oyster cages and exemplify how devastating predation can be in Hawaiian waters.

Brick (1970) documented 100% mortality by predatory *Stylochus* flat worms in all 150 oysters deployed at Coconut Island, Kaneohe Bay, O‘ahu within six weeks of having been transplanted. A second trial at the same location resulted in 40% mortality within two weeks, with empty shells filled with *Stylochus*. Similar to Brick (1970), this study recorded high mortality from flatworms from the genus *Stylochus* and predatory *Cymatium* snails as indicated by their presence inside the shell of newly killed oysters.

Comparable to previous research, this study found that triploid oysters suffered the least mortality throughout the growth trials. Other research has shown that triploid oysters exhibit decreased mortality under stressful conditions such as disease and summer mortality (Nell 2002, Gagnaire et al. 2006). This is due in part to the fact that triploids are generally sterile and therefore reallocate their reproductive energy from gametogenesis towards growth and survival (Gagnaire et al. 2006, Nell 2002).

Previous studies by Royer et al. (2006) in Normandy France showed high levels of epibionts colonizing their oysters and culture bags including sea squirts, barnacles, and polychaetes. During the Hilo Bay trials, cages and oysters regularly became encrusted with algae, epiphytic bryozoans, tunicates, and barnacles which may have reduced water flow and the quantity of available phytoplankton for oysters to feed on. Encrusting sponges resulted in complete suffocation of affected oysters leading to additional oyster mortality even when cages were cleaned every two weeks. The encrusting sponge also covered the cage reducing water flow which may have hampered growth rates. Sparks (1963) similarly documented encrusting and boring sponges covering oysters in Pearl Harbor. After six months in the water, oyster cages

became increasingly encrusted with algae, tunicates, encrusting sponges, oyster set, and tube worms during the two-week period between cleaning and sampling. As the long-line suspending the oyster cages became more incrusted, thorough cleaning of cages was necessitated, often requiring an entirely new cage to be swapped for the dirty one.

Climate change and summer mortality

Goulletquer et al. (1998) critically emphasized the importance of considering global environmental warming trends and how it relates to oyster mortality due to thermal and associated stressors. Aura (2016) further highlighted that trends in ocean temperature will result in reductions in suitable oyster culture sites. Natural oyster abundance, which is at 15% of historic levels, is hindered by habitat loss, eutrophication, and disease made worse by the effects of climate change (Aura et al. 2016). These trends have already been shown to cause increasing severity of summer mortality in oysters due to a culmination of environmental factors related to global warming and is only predicted to get worse in the coming years (Goulletquer et al. 1998). Climate change is also predicted to make many ideal culture sites less suitable as waters warm and environmental factors change (Aura et al. 2016) These factors become even more significant for Hawaiian Fishponds which have walls prone to being destroyed by large storms and shallow waters particularly sensitive to thermal fluctuations and changes in weather patterns (Keala et al. 2007).

Recommendations

With the implications of climate change affecting Hawai‘i, the grow-out window to avoid summer mortality is likely to get shorter as summer mortality increases in severity and duration forcing oyster farmers to out-plant later and harvest sooner if they are to avoid the devastating effects of summer mortality. One possible method to increase resistance to climate change

stressors is genetic selection of oysters resistant to summer mortality stressors. Selective breeding trials focusing on improving resistance to summer mortality resulted in significantly greater survival and yields during extreme summer mortality events, while not significantly effecting growth performance (Bédier et al. 2010).

New research by Chen et al. (2016) determined that the economic outlook of producing oysters in Hawaiian fishponds (100-200 thousand oysters per year) may not be profitable due a combination of factors including high labor costs and oyster mortality. The economic analysis also indicated that only a small reduction in oyster mortality rate, or slightly reduced labor would make the farm profitable. A large amount of manual labor is required to clean and maintain oyster stocks due to *P. websteri* and other pests in fishponds. Oyster mortality and labor could be greatly reduced by utilizing horizontal long lines that allow the oysters to be exposed to the air at low tide (Handley & Bergquist 1997, Mercado-Silva 2005, Royer et al. 2006). Future research in Hawai‘i should focus on investigating passive methods of controlling *P. websteri* and other pests, like (Handley & Bergquist 1997, Royer et al. 2006) methods of periodically exposing the oysters to air at low tide. One technique for reducing *P. websteri* infection and oyster mortality is with elevated off-bottom culture with regular exposure cycles, low stocking densities, and adequate flushing (Royer et al. 2006, Forrest et al. 2009). This practice usually utilizes two posts imbedded in the bottom of substrate and stringing a horizontal long line between them. The oyster cages or baskets are normally suspended from the long-line and are exposed at low tide to help with mitigating biofouling (Forrest et al. 2009). In addition to pest removal, these methods have already been widely used to also increase shelf life, shell density, and meat content of oysters (FAO 2014). Future research incorporating these methods may be essential for developing a sustainable and profitable oyster culture in Hawaiian fishponds. Chen et al. (2016)

also concluded that a threefold expansion in production would not incur such a large expansion in total production costs. This suggests that with current labor costs, there is an economy of scale at which point oyster production becomes profitable at level of about half a million oysters per year.

Our data suggest that there is a window between the months of September to July when oysters may be out-planted and then harvested at sellable commercial size in ten months or less. More research is needed to determine if growth and mortality trends recorded in this study are reflected in other fishponds and bays around the Hawaiian Islands. Seasonal variations in CI should also be analyzed. Utilizing all available data from this study, this study concludes that oyster cultivation is highly feasible in Hawai‘i but may be hampered due to a variety of site specific factors including temperature fluctuations, low phytoplankton concentration, and biofouling. These factors may be overcome by selection of appropriate growing location, deploying larger sized spat of a hardy variety such triploid or Midori oysters in the month of September so that they are ready to harvest in July before summer maximum temperatures in August. This study conducted research on varieties of *C. gigas* but future research should include other oyster species such as *C. virginica* (Eastern Oyster) and *C. sikamea* (Kumamoto Oyster). The results of this study suggest that the best grow out areas in Hawaii are in traditional Hawaiian fishpond that are protected from wave action and have high phytoplankton density. With the utilization of these ponds, Hawai‘i may see a renaissance in oyster-focused aquaculture in traditional Hawaiian fishponds, providing economic growth and sustainable careers for many current and future generations.

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