Integrating Biosystems to foster Sustainable Aquaculture:

Using Black Soldier Fly Larvae as Feed in Aquaponic Systems

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Final Thesis

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Executive Summary

Chapter 1 of this report examines the current state of aquaculture and identifies two major environmental concerns associated with it, namely nutrient pollution by effluent and use of fishmeal-based feed. Together, these concerns cast doubts on the sustainability of aquaculture with current techniques. The studies outlined herein address these issues through 1) aquaponic filtration of the aquaculture effluents and 2) the use of the Black Soldier Fly, *Hermetia illucens,* as an alternative to fishmeal in aquaculture feedstuffs. The introductory chapter examines the current state of aquaculture and makes the case that aquaponics is a viable option to manage aquaculture effluent in recirculating systems. Furthermore, Black Soldier Flies offer a locally sourced feed that is more sustainable, both economically and environmentally, than fish-meal based fish feed. Chapter 2 provides a detailed discussion of the logistics of aquaponics and Black Soldier Fly culture used in these studies. Separated as two stand-alone documents, Chapter 2 is developed as: i) Considerations of Aquaponics and ii) Techniques for Black Soldier Fly Culture with the intention of publication of each through the Agricultural Extension purview of the College of Tropical Agriculture and Human Resources of the University of Hawai'i Mānoa. These documents were written as a combination of literature review and case analysis of different systems built in support of these studies, and are intended for an audience of farmers, aquaculturalists, homeowners, researchers, investors, and others that are interested in sustainable aquaculture. Once the reader is convinced of both the importance and feasibility of both aquaponics and Black Soldier Fly Culture, the ultimate question is presented, "Are Black Soldier Flies a legitimate food for catfish?" Chapter 3 is the culminating research project, presented as a journal article, to answer this project's central question.

Keywords: aquaculture; aquaponic; black soldier fly; *Hermetia illucens; sustainable agriculture; integrated biosystem; alternative fish feed*

Unsustainable Aquaculture - Introduction to Issues and Overview of Solutions

Trends in Aquaculture: World, US, Hawai'i

Farm-raised seafood accounts for over one-third of the world's consumption, according to the 2012 report by the Food and Agriculture Organization (FAO) and is increasing steadily. Aquaculture, which has doubled globally in the last two decades, has the potential benefits of providing global food security while reducing pressure on the world's fisheries [1]. Currently, however, aquaculture often comes at the expense of several major negative environmental impacts. The largest problem is the unrestrained use, and subsequent depletion, of wild fish as aquafeed. The second problem is habitat modification and water pollution by the aquaculture effluent [2]. Also, intense aquaculture facilities are high-risk sources for the introduction of alien species and virulent diseases into the wild. Fortunately, mitigation solutions are available for each of these issues. Alternate feeds exist, as do effluent purifying methods. This paper will briefly review current aquaculture methods and trends, highlight the environmental concerns and provide an overview of emerging solutions to increase sustainability.

Aquaculture is an enormous industry, producing one-third of the seafood consumed world-wide. Globally, aquaculture produced 63 million metric tons, while global wild-caught production totaled 90 million metric tons in 2012. Of this staggering amount of aquaculture, half is freshwater fish, a quarter is aquatic plants and algae, and the remaining quarter is divided between crustaceans and mollusks. The largest subgroup within the freshwater fish is cyprinids, the catfish and carp family, grown mostly in ponds across Asia and the US. Salmonids grown in North and South America and tilapia farm-raised throughout the world provide further contributions to the freshwater fish totals. This is a multi-billion dollar business, and it is expanding rapidly. [1, 2].

In 2008, the United States lagged behind other countries in the use of aquaculture, farming only one-eighth of its seafood. In that year, 500,000 metric tons of aquatic animal products were farm-raised, while 4,350,000 tons were harvested from the wild. Catfish, mainly raised in the southern US, were the most widely cultivated species, accounting for two-thirds of the total aquaculture production. Further important freshwater species are tilapia, trout, crawfish, and hybrid striped bass; marine species include crustaceans such as shrimp and soft-shell crabs and mollusks such as oysters, clams and mussels. Very few aquatic plants are grown in the US outside of Hawai'i [1, 3, 4].

However, in Hawai'i algae is a $10.5 million a year industry, comprising half of all Hawaiian aquaculture industry which was worth a total of $21.3 million, according to the most recent Census of Agriculture conducted by the National Agricultural Statistics Service in 2008. Finfish and shellfish each contributed $2.4 million to this total. The majority of the aquaculture industry is centered in Hawai'i County, predominantly at the Natural Energy Laboratory of Hawai'i Authority facility (OTEC peninsula) in Kona, Hawai'i [5]. In Hawai'i, marine algae are cultured primarily for the production of carrageenans; the gel-producing species of *Gracilaria spp*., *Kappaphycus spp*. and *Eucheuma denticulatum* are the most widely cultivated [6]. Additionally, several species of macroalgae are grown as food, including *Gracilaria coronopifolia* and *Porphyra tenera*. Microalgae, such as the cyanobacteria *Spirulina spp*., are also cultured in Hawaiian ponds. Finfish cultures include moi, tilapia, catfish, carp, flounder, sturgeons, amberjack, snapper, and grouper. Shellfish cultures include marine shrimp, freshwater prawn, lobster and abalone [7, 8]. Brood-stock of the shrimp *Litopenaeus vanamei*, *L. stylirostris*, and *Penaeus monodon* are sold world-wide as they are resistant to some common diseases [9].

Types of Aquaculture

Aquaculture is classified into two main categories: intensive and extensive. Intensive culture is a highly managed system with large quantities of external inputs, resulting in high stocking density. Extensive culture is less managed with correspondingly lower stocking densities and slower growth rates. Intensive culture often has more detrimental environmental effects due to the higher inputs of feed, chemicals, and drugs which enter the environment through wastewater. Examples of extensive cultures include rafts of filter-feeding bivalves, ponds of crawfish or omnivorous finfish that do not require much additional feed, or algae. Conversely, most crustacean and carnivorous finfish are raised in intensive systems.

Worldwide, some of the most common techniques are pond cultures (which can be fresh, salt, or brackish water as well as extensive or intensive), recirculating tanks, and coastal ocean cages. Of these, pond culture is the most common method, and suitable species include finfish (carp, tilapia, catfish, and milkfish) and crustaceans (prawns, shrimp, and crawfish). The intensive culture of prawns in Kahuku and Ka'a'awa on the island of O'ahu exemplifies this technique, though extensive pond cultures elsewhere are also common. Recirculating tank culture, often used for species which are difficult to raise in ambient conditions, has the benefit of increased environmental controls but is more expensive and more labor intensive to manage [10]. Intensive by nature, ocean cage culture is widely used in Brazil, the US, and elsewhere to culture salmonids. Hawai'i is home to two open ocean cage culture facilities, which raise moi (Pacific Threadfin) and kahala (Amberjack). Just as the distinction between extensive and intensive is not always obvious, many of these techniques can be combined, blended, or outright discarded in favor of novel inventions.

Environmental Issues and Solutions: Feed

Aquaculture is established as a massive, world-wide industry; therefore, the environmental consequences can be significant and far-reaching. What does it take to raise these 20 million tons of freshwater fish? Large amounts of aquafeed are required and present the biggest obstacle to sustainability. Though somewhat counterintuitive, farm-raised fish are typically fed wild-caught fish [11]. Fishmeal is the most common ingredient in fish food because it is high in protein. From a conservation standpoint, however, this is discharging one debt by incurring another, with little or no net reduction in fisheries pressure [12, 13]. Wild stocks are still being exploited, and at alarmingly lower trophic levels. Most aquacultured species require supplemental feed, including finfish (except for a few species) and most crustaceans [14]. Therefore, according to the 2012 FAO report, up to 70% of the world aquaculture production requires feed [15]. This is misleading, however, because throughout Asia many of the pond farms of tilapia and carp are extensive, relying less on manufactured feed and instead, exploiting the omnivory of these species to eat whatever natural feed is available. Still, even in moderately intensive operations, the carnivorous fish and most crustaceans rely on supplemental aquafeeds, and whose primary ingredient is usually fish meal [2, 11], which puts a huge demand on forage fisheries. According to a 2007 survey conducted by Tacon, the aquaculture feed industry used 3,724,000 metric tons of fish meal (68% global production) and 825,000 tons of fish oil (88% global production) that year, which equated to 16.6 million tons wet weight of small pelagic forage fish [15]. Indeed, a portion of this quantity is derived from by-catch that is harvested anyway [14], but much of it originates from targeted fisheries for small, pelagic fish such as the Peruvian anchovy, *Engraulis ringens* [15]. This is the most heavily exploited fish in world history: 13,000,000 tons were harvested in 1971 and the catch has declined steadily since then to 8,000,000 in 2008 [1].

The problem is that as world-wide supplemental-feed aquaculture increases each year, the global stocks of forage fish are either steady or declining [2]. As an antithesis to sustainability, it is easy to predict that this trend cannot continue, and that alternate feeds are required to support the continued growth of aquaculture [16]. This is especially true in the face of global environmental change which is predicted to negatively influence forage fisheries [17, 18]. Supplemental-feed aquaculture using fish meal based feeds is therefore a direct threat to the conservation of wild-fish stocks.

Many studies have sought to replace fish meal and fish oil with other protein sources and have experienced varying success. Some tested methods include: beniseed and locust bean meals [19], soybean meal [20, 21], sunflower meal [22], meat industry by-products [23, 24], agricultural by-products [25, 26], highly fecund herbivorous fish such as sand smelt [27] or mosquito fish [28], the nitrogen-fixing water fern *Azolla spp*. [29-33], pre-pupae of the Black Soldier Fly, *Hermetia illucens* [34], and bacterial films grown on natural gas [35]. Generalizing these results, vegetable sources can be used to substitute some of the fish meal in manufactured feed, but after a certain point the growth and health of the fish become compromised. The challenge with vegetable meals and *Azolla spp*. is that carnivorous fish are less efficient at processing non-animal proteins, which lack essential amino acids and carbohydrates [2]. Agricultural and meat processing by-products are possible feed sources, but they can be either difficult or costly to obtain.

Of these emerging techniques, one of the most promising is the culture of Black Soldier Fly (BSF) larvae as fish feed, a technique that offers the additional benefit of reducing organic waste [36], and provides a potential solution for safe manure management, both human and animal [37]. BSF larvae eat butchering scraps, organic municipal waste, and livestock manure [38], as well as any organic waste from household kitchens. Once hatched, larvae eat for approximately six weeks until they become two centimeter pre-pupae, at which point they begin to crawl away from their feed, shifting from a feeding phase to a “wandering phase”. This wandering phase is dedicated to the search for a dry, safe place to metamorphose. In BSF culture, farmers can exploit this life history trait by providing a single path for the pre-pupae to leave the feeding bin so that the only exit leads into a collection container [39]. Collected larvae have excellent nutritional qualities, including on a dry-matter basis: 40-45% protein, 30-35% fat, 11-15% ash, 4.8-5.1% calcium, and 0.6% phosphorous, as well as beneficial amino acids and minerals [37, 40]. BSF larvae have been successfully fed to rainbow trout [40], catfish and tilapia [41] swine and poultry [37]. If the larvae are fed fish carcasses and butchering scraps, they can be high in omega-3 fatty acids [42], possibly improving the health and nutritional benefits of the fish. This also suggests that the BSF larvae could be grown on special diets to meet specific nutritional requirements of the target animal. Raising BSF larvae on manure and other waste is a value-added management system, which creates usable, salable animal feed where once there was only waste [43], while at the same time reducing harmful pathogens in the manure of poultry and swine [44, 45], and controlling houseflies, *Musca domestica*, in livestock facilities [46].

Environmental Issues and Solutions: Waste Water

In addition to the concern over the unsustainable nature of aquaculture feeds, the management of aquaculture wastewater is a serious issue. All of the nutrients from the feed that are not retained within the fishes' bodies are excreted and lost, and aquaculture facilities release this nutrient rich water into the environment, albeit in varying degrees. In 2004, the Environmental Protection Agency published guidelines regulating the effluent released by concentrated aquatic animal production facilities [47, 48]. These documents provide regulation of wastewater composition in conjunction with the US Clean Water Act, especially regarding the release of suspended solids, biochemical oxygen demand, nutrients, drugs, and other pollutants [48]. The FAO published similar guidelines in 2008.

These regulations are required because aquaculture effluent waters can be highly disruptive to the environment. Much of the fish feed is caught in off-shore waters, ergo, there is a net import of nutrients, namely nitrogen and phosphorous, into the discharge areas [48], which can cause eutrophication and hypoxia in watersheds and coastal areas [49]. This eutrophication can be exacerbated by the biochemical oxygen demand of the released chemicals [50-53] and/or subsequent algal and bacterial blooms [54]. Released nutrients contribute to macroalgae overgrowth of coral reefs, resulting in phase-shifts and other ecological disturbances [55, 56]. There have been world-wide catastrophic losses in biodiversity, species richness, and recreation value in waters polluted by nutrient excess. Additionally, suspended solids comprised of uneaten food and feces directly disrupt gill function [57] and increase turbidity, decreasing the depth to which sunlight can penetrate thereby affecting benthic flora [58].

Further concerns arise from the use of hormones, drugs, antibiotics, and other chemicals used for disease control and spawning management, which are released into the wastewater. The environmental effects of these pollutants are poorly understood [59, 60]. Moreover, fish meal is often high in heavy metals, which then accumulate downstream of the aquaculture facility [52]. Effects of these chemicals released into the environment include drug resistant diseases, mortality from toxins, and reproductive disruptions due to hormonal imbalances. A final disturbing aspect of effluent is the release into wild populations of disease-causing pathogens and parasites that flourish in the captive culture facilities [61, 62]. This is a particularly pressing concern if an endangered, wild population is located near a facility that grows a con-generic species. For example, endangered salmon and trout species in the Pacific northwest of the U.S. are living perilously close to cage cultures of salmon that are affected by a myriad of health issues [63]. Whirling disease is just one example of a captive disease that has spread to wild stocks from captive cultures [64].

In summary, most farms that raise carnivorous finfish or crustaceans import fish protein caught from declining wild stocks as manufactured aquafeed, the waste from which pollutes watersheds and coastal basins with nutrient rich waters filled with sediment, chemicals, diseases, and parasites. The solution to this pollution problem is reducing the total effluent volume while improving its quality. Nutrients and sediments can be sequestered by using a combination of plant uptake and mechanical separation. Many land-based aquaculture farms use mechanical techniques including settlement ponds, baffled separator tanks, and filters to catch the filterable (solid) waste [65]. Chemicals can be added to coagulate the solids to aid in separation [66-68]. The collected waste is then disposed of as soil amendments or fertilizers [69-71], or even feed for vermicomposting [72-74]. Dissolved nutrients are impractical, though possible, to filter [75, 76] and therefore bioremediation through primary production is the best method to strip the nutrients from the water; plants are used to filter the water of nutrients before the effluent is released into the watershed. Since nitrogen and phosphorous are two crucial nutrients for plant growth, and fish excrete these same nutrients, it can be said that plants utilize what fish excrete. Placing plants within the effluent stream reduces the volume of nutrients released into the watershed.

Decreasing the nutrient load in aquaculture effluent to meet environmental demands might be more welcomed if the effluent was used for the growth of plants with commercial value. Some techniques include using the effluent as irrigation water for dry-land crops [70, 77], culturing of marine algae in saltwater systems [78-81], and releasing the water through artificial wetlands [82-84]. A traditional Chinese method consists of growing crops around the edges of an aquaculture pond, irrigating them with the pond water, harvesting all of the fish at once and then cultivating another vegetable crop in the now-dry pond. An interesting project used human waste water to irrigate ornamental plants met with success as a method for small scale sewage management [85]. It may even be possible to formulate fish diets so precisely that the fish use all of the available nutrients, resulting in effluent devoid of pollutants [86]. A noteworthy solution called aquaponics is receiving renewed attention especially due to the work of Dr. James Rakocy at the University of the Virgin Islands [87].

Aquaponics is a value-added system in which a vegetable crop is hydroponically grown in recirculating aquaculture water. Recirculating aquaculture systems are typically limited by the toxic accumulation of nitrates from the oxidation of the ammonia in the fish waste, and often vent this nutrient-rich water into the environment. Instead, with a properly designed aquaponic system, the hydroponic plants are able to sequester these nutrients, especially nitrate and phosphate. One study showed that 69% of the nitrogen present in aquaculture tanks was transformed into plant biomass [88]. Besides the obvious economic benefit of a vegetable crop grown without additional irrigation or fertilizer, further environmental benefits include reduced water usage and total effluent volume due to fewer water changes. Also, lower effluent means that fewer antibiotics, drugs, and chemicals enter the environment. Profitability is a concern due to the higher maintenance, labor, and start-up costs; but with emerging, ingenious solutions, there is real economic feasibility [89-93]. It was found that the rate of return increased as the size of the farm increased, showing economy of scale by consolidating infrastructure and management costs [94]. However, aquaponics is limited to intensive, recirculating, freshwater aquaculture and alone aquaponics cannot address all of the sustainability issues surrounding aquaculture.

The current generation may be the last to eat wild-caught seafood, and aquaculture must be part of the answer if humans are to continue eating seafood at all.

The associated conservation concerns can be overcome by looking for natural solutions to man-made problems. One system's waste is another system's raw material: raising detritivores as fish feed and growing vegetables with fish waste water are just two methods that reduce pollution while reducing costs, both monetary and environmental. By using BSF as fish feed in an aquaponic system, a farmer would be able to harvest both fish and vegetables with minimal inputs. It is widely known that the pattern affects the process [95], and it is by creatively redesigning the patterns through integrated biosystems that we can optimize agricultural processes [96]. Moreover, any economic incentive that drives conservation-minded practices ought to be encouraged. Neither aquaponics nor BSF culture will solve the global environmental problems and neither is applicable to every situation, but these techniques offer additional tools for sustainable aquaculture. Aquaculture will continue to grow, and with continued research and inventions that include both economic and environmental dimensions, aquaculture will be able to develop more sustainably.

Techniques of Aquaponics

Introduction:

Aquaponics is a combination of aquaculture and hydroponics where vegetables are grown in fish-culture water. The word aquaponics can be translated to mean "aquaculture water, working to grow plants". This technique of modern agriculture has considerable potential to intensively culture fish and vegetables using a minimum of inputs, space, or water. Alone, aquaculture can have large environmental impacts [97], but aquaponics provides a technique to reduce some of these impacts while increasing profitability. However, intensive aquaponics requires the management of several separate organisms. All aspects of this artificial community need to be monitored and manipulated, including the fish, the plants, and the bacteria. Water is the unifying medium for an aquaponics system, and as such, water quality is critical. Furthermore, the water needs to be circulated through the system through relatively complex plumbing. Here an outline and brief discussions of important considerations are presented. Throughout, references are made to the aquaponic systems installed at the University of Hawai'i at Mānoa (UHM) campus which were used for various studies. Literature review and experience highlight key techniques to manage aquaponic systems at critical points.

Imagine aquaponics as a living system. The fish would be the stomach, eating and digesting the food. Nutrients dissolve in the water, the life blood of the system, and are circulated by the heart-pump through other organs carefully contained within PVC veins. Filters act like kidneys, collecting and consolidating poison from the blood stream to be excreted through occasional venting. Oxygenated gravel-bed lungs breathe air into the nutrient-rich blood where it is processed by microbes, much like gut flora, into a more usable form. Still dissolved in the water, these nutrients are used by plants to build structure, somewhat like hair or nails, to be trimmed off and harvested. This complex organism is also an environmental community in which the physical, biological, and chemical realms interacting to create a single cycle. Artificially constructed to meet the needs of the farmer, aquaponics intensifies and mimics a natural ecosystem. Like any community, ecosystem or living organism, damage to one part of the system imbalances the rest and can propagate systemically and cause catastrophic collapse. This paper treats aquaponics as individual modules in order to separate this complex system into a more structured discussion.

Farm Organization Module

Aquaculture farms require attention to the initial design to facilitate efficient workflow. Often, the aquaculture module is organized as 1) broodstock tanks used for breeding, 2) juvenile rearing tanks used to carefully manage the offspring until a critical size, and 3) grow-out tanks which house the majority of the fish. In production aquaponic systems, the grow-out fish tanks are connected hydroponic grow-beds. Water from the fish tank is filtered as it enters the sump, which is the lowest point of the system. The sump collects water as it runs downhill through return lines from higher tanks. Within the sump, a pump lifts the water through supply lines to the level of the hydroponic grow-beds. Within the grow-beds the water is biologically filtered and irrigates the soil-less media. Plants in the grow-beds strip the nitrogen from the water before the water is returned to the fish tank. Though many other designs are possible, it is important to ensure that any water loss is only manifest in the sump; the sump is the only tank that changes volume because the fish tank and grow-beds are controlled with standpipes. Furthermore, only one pump should be used in an aquaponic system which pumps the water to the highest point from where the water runs through the tanks and returns to the sump through gravity alone. Well designed facilities will also include centralized air supply lines, water lines, and electrical outlets.

It is important during the initial design to ensure that the components are correctly sized to one another. Each part must be proportional to the whole system so that the plant biomass is able to adequately filter the fish waste. Most literature that investigated the optimum feeding rates for aquaponics reports the amount of fish feed necessary to support one square meter of hydroponic plants, and then determines the number of fish required to eat that amount of feed based on feeding regime. Feeding regimes are discussed in more detail below, but generally between 1-5% of the fishes' total body weight is fed per day. Examples of experimental feed to area ratio are

Feed/Area g/m^2	Plant Species	Fish Species	Reference
15-42	Ipomea aquatica	Catfish	[98]
56	Lettuce	Tiliapia	[99]
60-100	X	Tilapia	[100]

Table 1: Component Ratios determined from literature values for the amount of feed added daily to an aquaponic system to support strong hydroponic growth.

reported in Table 1. During the UHM studies conducted between 2009 and 2013 all of the systems were maintained at a ratio approximately 50 grams of feed for every square meter of planted area. Higher ratios were occasionally maintained, but water quality deteriorated if the hydroponic module was not fully stocked with plants.

The following equations can be used to appropriately size the components on a theoretical basis. If the biomass of the fish can be determined through measurement or estimation, Equation 1, below, can be used:

1. (Number of Fish) * (Avg. Weight of 1 Fish) * (Feeding Regime) * (Area/Feed Ratio) = (Area of Hydroponics)

In the case that the number or weight of the fish is unavailable for a system that is already operational, the area for the hydroponics can be calculated on observed feeding using Equation 2:

2. (Weight of Daily Feed) * (Area/Feed Ratio) = (Area of Hydroponics)

The rationale behind the use of this ratio is to balance the aquaponic system. If too little fish feed is added to a system the plants will not thrive, but with too few plants in the system the water will accumulate nutrients and stress the fish. Perfect balance is unlikely because the ratios will continually change as biomass changes during growth and harvest. During the design phase it is better to err on the side of caution and install spare hydroponic grow-beds. The extra plants will ensure that the water is as clean as possible for the fish, while truly superfluous hydroponic area can remain unplanted until the fish biomass is great enough to support the full area.

Once the fish tanks and hydroponic grow-beds have been designed to the correct proportions, it becomes straightforward to connect the other parts of the system through the use of PVC plumbing and water pumps. An additional aspect is ensuring that there is adequate surface area for ample biological filtration. In some designs there is not enough room for necessary bacteria to live, and additional biofilters are required.

Redundancies and back-up systems must be developed throughout the design and implementation of any aquaculture system. Especially in the grow-out fish tank, it is critical that the water level is independent of leaks elsewhere in the system. There is zero-tolerance for losing the fish through water loss. If the pump is located directly in the grow-out tank, it must be lifted off the bottom or wired with a cut-off float-switch. A safer design is having the grow-out tank located higher in elevation to the sump tank that houses the pump. In this situation water overflows the grow-out tank through a standpipe which permanently sets a constant water height; any water loss would cause the sump to empty but not the fish tank. If air supply systems are used, which will be discussed below, a backup system needs to be available during loss-of-power events. One of the most effective designs has multiple, stand-alone aquaponic systems running in parallel. Although this design sacrifices some economy of scale, it allows greater control and can contain failures to one compartment and prevent catastrophic, complete losses that could be caused by leaks or disease. Dr. Rakocy provides a 10 part guide that serves as a planning document to help during the design phase [100] and highlights the importance of proportional design from the initiation of the project.

Aquaculture Module

Fish Selection

The fish species should be selected based on local environmental conditions and local market demand. Generally the fish need to tolerate stressful conditions such as crowded tanks, low dissolved oxygen levels, pH fluctuations, and spikes in ammonia. Though aquaponics minimizes these stressors, it is convenient to have a fish species that is sturdy.

Tilapia, *Oreochromis spp.*, are a common choice of fish in aquaponic systems primarily because tilapia tolerate poor environmental conditions. Equally important is the fact that there is a market for tilapia which are prized for their white, mild, and flakey flesh. Finally, tilapia have a fast growth rate and process large quantities of fish feed. The most widely used tilapia species are Nile Tilapia, *O. niloticus* , including the Black, Red and White varieties, the Mozambique Tilapia, *O. mossambicus,* which includes the Hawaiian Golden Tilapia, and the Blue Tilapia, *O. aureus*, also known as the Israeli Tilapia. Most of these agriculturally important species exist as hybrids among various other species. The Rocky Mountain White, *O. niloticus x aureus*, is a popular variety because it tolerates lower temperatures. Tilapia are a warm-weather species, preferring temperatures between 20-30ºC (68-86ºF), and as such their culture is limited to warm climates. However, tilapia aquaculture has been successful in temperate climates when coupled with a source of heated water, such as electrical power plants. Tilapia males grow faster than females, which is one reason that large operations cultivate monosex cultures. In addition to the faster growth rate, when the sexes are kept separate production can be higher because fish are not using energy on sexual development or courtship behavior. Industrially, monosex culture is achieved by feeding juvenile fish steroids to masculinize all of the fish, despite concerns of human health and environmental consequences. Alternatively, tilapia can be manually separated by sex and kept in segregated tanks.

The Hong Kong catfish, *Clarias fuscus*, is another attractive choice for aquaponics for the same reasons as tilapia. Together, these two species were cultured during the course of the UHM studies. As air-breathers, catfish are especially well suited to low oxygen situations and were observed to survive conditions that killed tilapia.

Other fish species used in aquaponics include Silver Perch, *Bidyanus bidyanus*, Pacu, *Piaractus brachypomus,* Swai, *Pangasianodon hypoththalmus,* Rainbow Trout, *Oncorhynchus mykiss,* and ornamental Koi, *Cyprinus carpio haematopterus.* Rainbow trout were cultured during the UHM studies but were not used for aquaponics because the ambient air temperature in Mānoa, O'ahu was too high for trout so they were maintained in an air-conditioned lab separate from the aquaponics.

Fish Breeding

Broodstock is not harvested as a crop because it is the most valuable life stage of the fish. Broodstock should be kept in tanks separate from the main grow-out tanks, preferably in a stand-alone aquaponic system, and managed independently. The motive of this broodstock management method is to keep the fish safe and secure, well fed and reproductively active.

Intense discussion of breeding techniques is outside the scope of this paper, but extensive literature is available. Briefly, tilapia will spontaneously spawn when both sexes are held together in tanks appropriate environmental conditions. These conditions include appropriate temperature, substrate, and water quality; tilapia generally prefer warm, green-water culture with a gravel bottom for breeding. Refer to [102] for an in depth discussion of tilapia breeding. Unlike tilapia, catfish do not spontaneously spawn, and require more involved breeding techniques. At the appropriate time, sexually mature broodstock are injected with human growth hormone which induces the final stages of gamete production. The eggs and sperm are expressed from the fish into a container and mixed to ensure fertilization before being transferred to a nursery and juvenile grow-out tanks. See [101] for full methods. During the course of the UHM studies, catfish were successfully spawned four times to stock the aquaponic systems and to monitor growth using different diets. Several cohorts of tilapia spawned incidentally in the grow-out tanks, but the fry were often eaten.

Juveniles and fry are maintained in small tanks, coddled with high quality feed and pristine environmental conditions. Fry must be segregated from larger cohorts to prevent cannibalism. Proper broodstock management and carefully breeding ensures the availability of juvenile fish to replace the harvested adults. Additionally, juveniles are often available at agriculture distribution centers, and can offer an additional market for farms with excess production.

Fish Feed

The major input to aquaponics is the fish feed, which can constitute up to 60% of the cost of raising fish. High quality feed provides a balanced diet with all essential amino acids and nutrients, while dietary imbalance can cause a drastic decrease in growth. Feed requirements are available for all major aquaculture species. A review of important considerations is provided by Glencross [103].

Traditional feeds are based on fishmeal, which is a processed product of wild-caught fish. Usually fish meal is made from small, pelagic fish such as anchovy harvested through targeted purse-seining. These fish are dehydrated and ground into meal as the primary protein source in aquaculture feed [14]. There are grave environmental concerns regarding the over-exploitation of global fisheries, and fish meal is seen as an unsustainable aspect of aquaculture [2]. Fortunately, alternative options exist. Many other products have been substituted for fishmeal, but generally the protein content is either not high enough or the amino acid profile is unbalanced. Therefore, many of the alternative feeds are not economical for the farmer because either the fish grow slower or the feed is expensive. However, in aquaponics the fish growth is not the only source of income, and instead the hydroponic plants provide the bulk of sales. The feed is merely the raw supply of nutrients for the plants. Ergo, net profits could be maximized with a feed that is inexpensive, even if it results in slow fish growth, by providing adequate nutrients for the hydroponic module.

One such feed is the Black Soldier Fly, *Hermetia illucens,* which is an insect larvae whose larvae can be raised on organic waste, and have excellent nutritional qualities for aquaculture feed [41, 43]. One caution is that unprocessed Black Soldier Flies have a chitinous shell that is completely indigestible to fish. The chitin acts as a non-nutritional filler and takes away from the quality of the feed potentially resulting in the slower growth of the fish in the UHM studies. Processing the Black Soldier Flies through dehydration, milling, and reformulation increases their potential value as a feed but increases the costs. Additionally, the shell is more likely to clog filters than traditional pellets. There has been some success in the UHM studies of feeding the shells to crustaceans which possess the digestive enzymes to process chitin. Even so, Black Soldier Flies are simple and inexpensive to raise, can be produced locally, and elicit positive growth from fish, thereby reducing both the monetary and environmental costs of an aquaponic venture.

In addition to feed composition, it is important to consider the daily ration and feeding regime. This refers to how much feed is presented to the fish each day. Generally, feeding regimes are calculated as a percent of the fishes' body weight. Typical feeding regimes range from 1-5% of total body weight per day. Younger fish eat proportionately more than larger fish. Again, there is extensive literature available regarding all the important aquaculture species. Feed is often presented *ad libitum*, which means as much food as the fish will eat in a set amount of time, usually 10-15 minutes. An *ad libitum* diet ensures that the fish are not being limited by the amount of food, but this method can be inefficient because some of the feed is wasted. At the end of each feeding session uneaten food should be removed and quantified so that the farmer can monitor the amount of food eaten. Observing changes in eating patterns can provide information as to the health and growth of the fish. Additionally, the amount of food entering a system is the critical value that needs to be known to manage the hydroponics module and water quality effectively.

Disease Management

Many aquaponic farmers grow fish in high densities to maximize the nutrients available for plant culture, but these crowded conditions can be stressful for fish. At the same time, the hydroponic module of aquaponics is a massive filter and as such, fish experience clean, oxygenated water. The most important aspect of managing disease is prevention. Always obtain the best available broodstock. Some broodstock are known to be resistant to some diseases. Best practice is to always quarantine new fish before adding them to a system. The most common treatments for parasites such as Ich, *Ichthyophthirius mulitfiliis*, are either salt or chemicals, both contraindicated for aquaponics because of the plants. Infected fish, if noticed, should be removed and treated in a separate treatment tank, and culled if necessary. Antibiotics can be used for bacterial infections, but should be avoided if possible. It is always a good strategy to have separate, disconnected aquaponic systems running in parallel. A concern specific to Hawai'i are pathogenic bacteria in the Rickettsia family. Referred to as "the disease", a Rickettsia-like organism (RLO) struck Hawaiian tilapia culture in the early 1990's [104, 105]. To date there has been no treatment discovered. Even so, surviving broodstock showed increased resistance to this disease and RLO has since stopped being an epidemic on established farms.

Water Quality Module

Introduction

Water is the essence of aquaponics, and as such, the importance of water quality cannot be overstated. The fish and the plants are entirely dependent on water for their survival, so monitoring the water quality is essential. Dissolved oxygen, nitrogen levels and pH are the most important parameters for the health of the system. Furthermore, filtration is essential to recirculating aquaculture, and it can be divided into two categories, mechanical and biological. Mechanical filtration is the process of sequestering particulate wastes from the water. Biological filtration is the process of converting the raw fish waste into a form more usable by plants and less toxic to the fish. Finally, the water needs to be circulated between the fish and the plants (typically through pipes and powered by an electric pump).

Dissolved Oxygen

Dissolved oxygen (DO) is a measure of the amount of oxygen in the water. Adequate DO is needed for the health of the entire system; fish, bacteria, and plant roots all require DO for respiration. In aquaponics it is suggested that DO is at least 5 mg / L [100]. DO decreases as the biomass of fish and plants increases and as the system collects organic wastes [98]. Furthermore, any algae present in the system will use DO during nighttime hours resulting in a reduction of DO, a process similar to eutrophication in natural waters. To provide enough DO for an intensive operation supplemental aeration is required: this DO can be achieved with the use of air pumps or blowers connected to diffusers. Cold water holds more DO than warm water, so proper aeration becomes more important as temperature rises. Though some species of fish, including tilapia and catfish, can survive low DO levels, supplemental DO should be added to support necessary microbial activity and plant root respiration.

pH

Recirculating aquaculture generally experiences low pH. Conditions become more acidic because fish respire carbon dioxide which reacts with water to become carbonic acid. Additionally, the oxidation of ammonia to nitrate liberates hydrogen ions. Together, these chemical reactions cause lower pH, causing aquaponic systems to become more acidic over time. pH is an essential water chemistry property to measure because it can affect the fish, the bacteria, and the plants. A pH between 6.5 and 7 is recommended for aquaponics. A pH outside of this range can cause the plants to experience nutrient lock, which refers to a situation where a nutrient is present at desired concentration, but the plant is unable to use it. This occurs according to the rules of acid/base chemistry and how plant roots absorb charged particles. Also, ferric iron is insoluble in neutral and basic conditions, so iron deficiencies can occur if the pH is too high. Fish prefer a neutral pH, but accept pH levels between 6 and 8. Nitrifying bacteria work more quickly at pH between 8-8.5 [106]. The recommended balance is a pH between 6.5 and 7.

To mitigate low pH, the literature suggests alternating additions of calcium hydroxide and potassium hydroxide [100]. The hydroxide ions neutralize free hydrogen ions, and the liberated earth metals are utilized as nutrients by the plants. Preliminary data from the UHM studies show that the addition of coral sand can have a buffering effect on the pH. Coral sand, calcium carbonate, dissolves in acidic conditions. The basic carbonate ion is released and neutralizes the acidic conditions, and the calcium ion is released and becomes available to the plants. The theory is that the calcium carbonate acts as a buffer, remaining stable at pH levels greater than or equal to 7, and that it will not dissolve unless the system is acidic. Therefore, coral sand will never cause the system to become basic, which can be more dangerous to the system's health than acidic conditions.

Daily fluctuations of pH can result when algae is present in the water, which is caused by algae either producing or consuming aqueous carbonic acid through respiration or photosynthesis, respectively. In green-water cultures, unicellular algae in the grow-out tank cause daily fluctuations but remain balanced over time. However, in aquaponics, algae are out-competed for nutrients by the hydroponic plants, which use gaseous carbon dioxide rather than aqueous carbonic acid, and therefore do not raise the pH. Basic conditions can occur when an abundance of algae in the system absorbs dissolved carbonic acid during photosynthesis. A trend of increasing pH suggests that algae are absorbing all of the carbon dioxide produced by the fish, which can be remedied by decreasing photosynthesis or increasing respiration with more fish [107]. This is one reason that fish tanks should be shaded and water should not be exposed to direct light.

Mechanical Filtration:

Dissolved nitrogen is welcomed in aquaponics since it is the primary nutrient for the plants. However, solid wastes comprised of feces and uneaten food must be filtered and removed. These wastes can clog the system, especially cinder beds, filters, and pipes, thereby creating anoxic zones and disrupted water flow. Anaerobic bacteria can develop in inadequately oxygenated filters. These bacteria can cause denitrification, where nitrate is converted to nitrogen gas, which becomes completely inaccessible to plants and escapes into the atmosphere. Denitrification is inconsistent with the goal of aquaponics, which is to reclaim the waste nitrogen in a plant crop. Also, undesirable bacterial activity can occur within these anoxic zones and release toxic hydrogen sulfide into the water. A healthy aquaponics system does not have beds clogged with particulate wastes.

Mechanical filters must be installed between the fish grow-out tanks and the biological filter. The filter material can be as simple as green scrub pads, standard aquaculture filters, shade cloth, or coated tangled plastic. These small filters need to be rinsed often. A more efficient approach for larger operations is to install a baffle filter, settling tank, or clarifier. These types of filter use the hydrodynamic principle that slower moving water cannot carry as much particulate material as fast moving water. A baffle is a container that forces the water through a series of bends slowing it down and shedding its particulates at each bend. Similarly, swirl filters use a vortex to consolidate particulates in the center of the vortex. Wherever the solid waste accumulates, there should be an easy method to remove it to prevent the filter from becoming anoxic. Ideally a single valve is installed so that it may be opened for the waste to run out. Removed wastes may be directed into the landscape as a fertilizer for other crops, or consolidated and used as a soil amendment.

Red worms may be added to particulate grow-beds to eat the solid wastes that escape the filters. Earthworms eat unfiltered solids, as well as dead leaves and roots. Earthworm waste dissolves more completely, enters the nutrient cycle, and is more readily available to the plants. Unfortunately, red worms have difficulty in the cold, flooded, sharp cinders and alone they are not enough to remove solid waste from the system.

Often in aquaponics the hydroponic grow-beds inappropriately become mechanical filters. Grow-beds filled with aggregate strain the water and collect particulates, and float-beds are generally long with slow current and act like settling basins. In this situation, the hydroponic grow-beds need to be frequently drained and rinsed, which can disrupt crop cycles and add unneeded labor hours. It is a better design to separate these functional parts and install dedicated mechanical filtration.

Biological Filtration:

Biological filtration is the process of converting raw fish waste into a more useful form. Biological filtration transforms ammonia into nitrate through the action of bacterial oxidation, a process called nitrification. Ammonia (NH_3) is a byproduct of protein metabolism, and is excreted by the fish through the gills. This ammonia dissolves in the culture water and is not captured by the mechanical filter. *Nitrosomonas* bacteria, naturally present throughout the system but concentrated in the nooks and crannies of the biofilter, are able to obtain energy by oxidizing the ammonia molecule. The by-products of the *Nitrosomonas* metabolism are nitrite ($2NO_2^-$), water (H_2O), and hydrogen ions (H^+). Nitrite is further oxidized by a different genus of bacteria, the *Nitrobacter*, into nitrate (NO_3^-). The final product, nitrate, is more usable by plants and less toxic to the fish than either ammonia or nitrite. Essentially, biological filtration in aquaponics is achieved by intensifying the natural nitrogen/bacteria cycle by increasing area available for the beneficial bacteria to colonize. The summary equations are shown below:

$$2NH_3 + 2O_2 + \textit{Nitrosomonas} \Rightarrow 2NO_2^- + 2H_2O + 2H^+$$

$$2NO_2^- + O_2 + \textit{Nitrobacter} \Rightarrow 2NO_3^-$$

There are two essential points concluded by looking at these equations. First, both reactions require oxygen as a reactant. If there is inadequate dissolved oxygen the reactions will not occur. This is the reason that biofilters are often oxygenated, whether it is through air stones, rotation through the air, or a fill-and-drain/ebb-and-flow cycle. Fill-and-drain, aggregate grow-beds are a popular method of ensuring adequate oxygen for the biofilter and are discussed below. Additional DO is supplied to the biofilter from any type of the aeration used in the fish tanks. Second, both reactions are reduction-oxidation reactions (RedOx), and overall hydrogen ions are liberated from the ammonia. This means that all recirculating systems will become more acidic over time. The implications and management of this constantly dropping pH are discussed in the previous section.

A large amount of ammonia needs a large population of bacteria to ensure complete nitrification into nitrate. This population of bacteria needs adequate space to live. The interior surface area of all of the tanks, pipes, roots, and grow media will be covered by these bacteria. However, systems with high fish density require additional surface area. The aquarium industry offers biofilters that are simply inert materials with a high surface area/volume ratio, examples of which include Bioballs™ and expanded clay tubes. PVC shavings, 6-pack ring holders, and volcanic cinders function just as well and are less expensive. There is research being done currently on the minimum amount of biofiltration necessary for aquaponic systems. The calculation to size the biofilter is driven by the amount of feed being added to the tank. A few large fish eat the same amount as many small fish, so it is essential to size the biofilter according to the expected daily ration of food. Generally, the biofilter should be oversized to ensure maximum nitrification.

[Ammonia]/[Ammonium]

Ammonia, NH_3, is a base, or proton-acceptor. Ammonia is highly miscible in water, and readily becomes protonated in the presence of an acid. Temperature, pH, and salinity affect the protonation of ammonia into ammonium, or NH_4^+. Ammonia, the un-ionized form, is more toxic to fish than ammonium. However, there is not a readily available test for either individually. Instead, colorimetric test kits measure Total Ammonia Nitrogen (TAN), which is the sum of NH_3 + NH_4^+. The manager needs to calculate the ratio based on temperature and pH. Warm, basic conditions cause more free ammonia, which is more stressful to fish [108]. An empirical study by Thurston [109] provides tabulated data for the ratio of unionized ammonia for every temperature and pH.

With a properly functioning, adequately sized biofilter in place, the TAN will not accumulate, having been oxidized by bacteria and used by the plants. However, even in a well-cycling tank, the TAN can spike after early morning feedings. This spike of TAN can overwhelm the biofilter, and can build up in the aquaculture tank before enough water can be recirculated; high residency time in the fish tank can allow a temporary increase in ammonia. If the entire system is neutral or acidic this spike is inconsequential. However, if the tank is even a little alkaline that spike of TAN is really a spike in unionized ammonia, and can be stressful to the system which highlights the importance of monitoring and managing pH in an aquaponics system and implementing a pH buffer material.

Water Management: Movement

The water needs to be circulated throughout an aquaponic system to connect the fish water to the plants. Most often, an appropriately-sized, electric pump is used. If faced with a choice, an oversized pump is better than an undersized pump. All excess head pressure from the pump should to be shunted to another tank. There is no reason to restrict the flow from the pump, which is difficult to accomplish and can be damaging to the system. Instead, allow excess pressure to return to the fish tank which has a benefit of further water movement and aeration for the fish.

One design put forth by Ako [110] does not use a water pump at all, instead relying on a human to transfer water every day from the fish tank to a raft type grow-bed. However, aeration is still required to keep DO levels high enough for the bacteria. Once the ammonia has been oxidized into nitrate in a biofilter, the processed water can then be used in non-recirculating (aka stagnant or static) hydroponics. This method requires very low maintenance, and has been used successfully in UHM studies to grow basil, green onions, parsley, and mint in a minimally-managed, "kitchen-garden" styled system. Moreover, this method can be employed in regions with limited access to water pumps or electricity.

Water Management: Siphons

Plumbing design can cause siphons within the system, which are sometimes desired and sometimes accidental. An autosiphon is a deliberate plumbing design that is used to control an ebb-and-flow type of grow-bed. Water constantly flows into a grow-bed until it forces an air bubble through the drain, which triggers a siphon that drains the tank faster than water is flowing in. Once the tank is empty, an air bubble is sucked into the drain, thereby plugging the drain and breaking the siphon. Then, the tank begins to fill again until the water level is higher than the bubble, thereby triggering the siphon and the tank flushes again. There are two main designs of autosiphons. The first design is the “Bell Autosiphon” which uses an internal standpipe with a larger diameter cap that reaches to the bottom of the grow-bed: as the water in the bed exceeds the internal standpipe, the air bubble within the cap is forced through the standpipe initiating the siphon. Once the bed is emptied to the level of the cap a new air bubble plugs the drain. For complete discussion see [111]. The second type of siphon is a “Looped Autosiphon”, which is as simple as creating a loop in the flexible hose used for the return line from the grow-bed. The siphon starts when the water level in the tank is higher than the highest loop of the hose. Once the tank is drained the entire hose empties and breaks the siphon. For all siphons it is important to have control over the inflow rate. Generally, the drain rate of the siphon needs to be greater than the inflow rate. However, the inflow rate has to be great enough to trigger the siphon. Once built, the only way to control siphons is by altering the inflow rate. Each autosiphon grow-bed should be controlled individually. Autosiphons are notoriously inconsistent and unstable. Often, poor drainage caused by particulate waste will prevent the autosiphon from operating properly.

Accidental siphons can occur when a drain pipe is submerged into the receiving tank. This can cause losses of water and has the potential to drain the source tank. To prevent this mistake the aquaponic design should ensure that all water lines have a gap between the pipe and the subsequent tank; waterfalls between pipes and tanks serve as anti-siphons. Also, foolproof redundancies in the system insulate the fish tank from any plumbing mistakes downstream.

Water Management: Drains

Drains can become clogged with fish waste and roots, which can in turn cause overflowing and loss of water. This undesirable situation can be mitigated by using large diameter drain pipe. It is prudent to oversize all drains. Additionally, it is important to ensure that all drains be as accessible as possible for cleanout. French drains can be used along the bottom of particulate grow-beds to ensure equitable drainage along the entirety of the bed. These French drains would be periodically "snaked" with a plumber's tool and also flushed with high pressure hose water to clear obstructions.

Water Management: Consumption

Aquaponics uses less water than traditional soil gardening. Very little water is lost from evaporation or watering the soil; the only water used is what the plants transpire. One study found that an aquaponics system only lost 1.4% of the total system's water each day in Saudi Arabia [99]. This highlights the water efficiency of aquaponic systems, and supports the use of aquaponics in sustainable farming applications, especially as global water reserves are facing increasing pressure.

Hydroponics Module

Introduction

Aquaponics combines nutrient-rich aquaculture water with hydroponic growing techniques. Hydroponics is a technique used to grow plants without soil, and it is solely through the water that the plants obtain their essential nutrients. Adequate knowledge of general hydroponic techniques is required for successful aquaponic system management. There are many hydroponic system designs, some of which are more amenable to aquaponics than others. Given that the plants obtain all of their nutrients directly from the water, proper management of nutrient concentrations is essential for efficient plant growth.

Grow-bed Designs:

The hydroponic modules of aquaponic systems are generally designed as nutrient film technique, deep raft, or aggregate beds. These variations of hydroponic grow-beds are location where the plants actually grow, and each design has advantages and disadvantages. Although other techniques exist within the hydroponic literature, they are less suited to aquaponics and are not discussed here. Lennard [112] determined that grow-bed designs had no effect on fish growth, but that particulate beds elicited more vegetable growth than deep raft cultures, which were in turn better than the thin nutrient film technique. It is the opinion of the author that a particulate bed, on an ebb-and-flow cycle, that drains into a deep raft basin is the optimal choice for aquaponics.

<u>Nutrient Film Technique (NFT):</u> In this technique the hydroponic solution, or filtered aquaculture water, is run at a slow velocity and low volume through hollow tubes into which the plants have been inserted [113]. The plants are held in net-pots or other perforated containers with a minimum of media, and the bare roots extend into the pipe creating a mat. Usually the growing tubes are made from PVC pipe or vinyl fence posts and a hole-saw is used to drill holes slightly smaller than the net- pots. The diameter of the growing tube's cross-section is typically between four and six inches. The vinyl pipes are preferred because the square cross-section gives more surface area to the roots. Many of these growing tubes are aligned in parallel at a slight angle. The nutrient solution is delivered through a manifold at the high end, and collected through a communal drain to return to the sump. Benefits of this system are that the roots are never exposed to light, yet the humid conditions within the pipe facilitate gas exchange. NFT systems are easy to clean and sterilize, and do not collect solid wastes. It is possible that the growing tubes clog with excessive roots. Perhaps more importantly, on a large scale this method is likely to become over-designed and too costly. Each tube requires several PVC fittings for the distribution and drain manifolds. This technique may be better suited for plants with more complex nutrient requirements, such as

squash, peppers, cannabis, or other flowers, because supplements can be easily added to the water and precisely targeted.

Deep Raft: This technique suspends rafts of plants over a basin of slow moving, nutrient-rich water, with the bare roots of the plants extending down into it. This is the fastest, easiest, and cheapest method for large scale production [114]. Most commonly, the rafts are thick Styrofoam sheets, but plywood can be used if suspended above the water. In fact, some experts recommend suspending all rafts 2-3" above the water, citing the positive effect of increased air at the root level on plant growth and health. Holes are cut into the raft using a hole-saw slightly smaller than a net-pot's rim. Seedlings are inserted into net-pots containing inert medium and placed so that the bottom of the net-pot extends past the raft into the water. The basin that holds the water is simply constructed by using lumber to create the sides and bottom and using a pond liner for waterproofing. Often, 2" x 6" boards are used for the sides and ¾" plywood used for the bottom. Basins are generally lifted up off the ground for easier maintenance, and can be supported with cinderblock or lumber legs. The water is kept at a constant depth between 6"-12" by using a standpipe. It is convenient to have these basins designed such that the rafts do not need to be cut during installation. Standard Styrofoam and plywood are typically sold in 4' x 8' sections. Ergo, raft beds are usually 4' across and a multiple of 8' feet long. This method is especially well-suited for medium to large scale cultivation of fast-turnover crops, such as lettuce, arugula, Asian cabbages, chard, kale, etc. Tall crops can cause undue torques on the Styrofoam sheets. One noteworthy drawback to a deep raft system is that both Styrofoam and plywood may contain toxins which may leach into the culture water, though this has not been thoroughly investigated. Also, the deep water basin can harbor pests including arthropods, isopods, amphipods, and gastropods. Most of these are inconsequential, but some gastropods will eat the exposed roots. If light enters the basin, either through gaps between the sheets or through the net-pot holes, algae and cyanobacteria can coat the roots and compete for energy. Also, because the media is so close to the water, it has a tendency to stay wet which can cause fungal and bacterial problems especially on the stems.

Particulate: With this technique plants are grown in a bed of particulate media irrigated with nutrient-rich water. The most common media include sand, cinder, gravel, and expanded clay aggregate. The bed itself can be constructed in much the same was as the Deep Raft basins by using lumber and pond liner. Alternatively, recycled containers such as polyethylene barrels, water tanks, plastic construction tubs, repurposed fixtures like bathtubs or sinks, or fiberglass vessels can be used. Also, particulate media is especially beneficial for tall plants or long-lived plants that need further support for their roots than deep raft culture can provide. Particulate beds function as both mechanical and biological filters as well as being an area for plants to grow. Any good particulate media will be extremely porous, which ensures a high surface area to volume ratio. This means that for a given volume there is actually more area for the nitrifying bacteria to colonize. For example, volcanic cinders are better than sand or gravel. Also, porous media facilitates gas exchange through the roots. Particulate beds are commonly plumbed on an ebb and flow cycle either by using timers, float valves on the pumps, or autosiphons [111]. This cyclical ebbing and flowing is intended to deliver air deep into the grow-bed, providing opportunity for gas exchange for both the bacteria and the roots. However, Lennard [115] investigated the differences between an ebb-and-flow versus a constant flow design in a gravel filled grow-bed. It was shown that a constant flow design had better pH buffering capacity and resulted in higher vegetable yield, and no differences were seen between treatments on the fish growth. Due to the fact that particulate beds function as mechanical filters, the beds can become clogged with solid fish waste. This causes anoxic zones, inequitable nutrient delivery, difficulties managing water flow and can lead to overflow situations. Other drawbacks of the particulate media include the increased weight, in the case of rock, and cost, in the case of expanded clay aggregate. Additionally, cinders are sharp and can be hard on equipment and plants. Additional research is needed in this aspect of aquaponics, and innovative approaches are being investigated. For example, disposable organic media, such as straw or mulch, can act as a particulate media and then be replaced with each harvest.

Nutrients

In aquaponics, all of the nutrients that the plants need are contained within the culture water. Unlike plants grown in soil, hydroponically grown plants are entirely dependent on the nutrient inputs added by the farmer. Nutrient deficiencies manifest quickly. However, it is relatively simple to adjust the nutrient levels because amendments readily dissolve and are available from dedicated hydroponic stores. Unfortunately, some amendments are expensive, and some cannot be used because it could negatively affect the fish and/or bacteria. Each of the important nutrients is discussed, including the biological function, signs of deficiencies, and source of additions. Much of this information is synthesized from observation and expert opinion, but McCauly [116] offers a useful tool to identify nutrient deficiencies. Also, Seawright [117, 118] quantified the plant uptake of nutrients from aquaculture water to determine limiting nutrients. Overall, the nutrients produced by the fish match the requirements of the plants fairly well; however, over time nutrient deficiencies can develop and must be supplemented with external inputs available from hydroponic supply stores. These elements and micronutrients are used by plants in small amounts, but are nonetheless essential to plant growth [119]. An alternative to processed, ready-made supplements can be found in worm castings or vermicompost tea [120]. The most commonly added amendments in aquaponics are iron, potassium, and calcium.

Macronutrients

Nitrogen (N) is the basis of all proteins. As such, nitrogen is the most common element in a plant after carbon and oxygen, both of which are obtained from the air. Nitrogen is therefore the key element in a hydroponic nutrient solution and serves as an easy to measure proxy indicator for other nutrients. Usually, dissolved nitrogen is in the form of nitrate, NO_3^-. Nitrogen deficiencies are obvious, and include yellowing of older leaves, thin stems, and poor vigor. Since nitrogen can be reallocated within plant tissues, it can be mobilized from older leaves and delivered to new growth which is why deficiencies are seen in older growth. An overabundance of nitrogen can cause excess vegetative growth, resulting in lush, soft plants susceptible to disease and insect damage, as well as causing difficulties in flower and fruit set. In strict hydroponics nitrogen is added through addition of calcium nitrate or potassium nitrate (saltpeter). The target of aquaponics is to use nitrate produced within the aquaculture module to fulfill the nitrogen demand of the plants.

Phosphorous (P) is used by plants as the backbone of DNA, as a structural component of the phospholipid membranes, and as ATP. Phosphorous deficiencies commonly cause poor root development because energy cannot be properly transported through the plant. Older leaves can appear dull green or even purplish brown. In strict hydroponics phosphorous is added as monopotassium phosphate. Other species of phosphorous are available but can be dangerous for the system.

Potassium (K) is used for cell signaling via controlled ion flow through membranes. Potassium also controls stomatic opening, and is involved in flower and fruit set. Potassium deficiency manifests as burned spots in older leaves and poor plant vigor and turgor. In strict hydroponics potassium is added as monopotassium phosphate or potassium nitrate. If neither phosphorous or nitrate need to be adjusted then potassium sulphate can be used. In aquaponics, potassium is often lacking because there is not enough of it in the feeds; it can be supplemented with potassium hydroxide, a strong base, which will also raise the pH.

Magnesium (Mg) is the center electron acceptor in chlorophyll molecules and is a key element in photosynthesis. Deficiencies can be seen as yellowing of leaves between the veins especially in older parts of the plant. Hydroponic solutions use magnesium sulphate (Epsom salts) to supply magnesium. Though sometimes the concentration of magnesium is low in aquaponics, it does not appear to be a limiting nutrient and addition of magnesium to the system is generally unnecessary.

Calcium (Ca) is used as a structural component of both cell walls and cell membranes. Deficiencies are common in hydroponics and are always apparent in the newest growth because calcium is immobile within the plant. Tip burn of lettuces and blossom-end rot of tomatoes and zucchinis are examples. Calcium can only be transported through active xylem transpiration so when conditions are too humid, calcium can be available but locked-out because the plants are not transpiring. Increasing air flow with vents or fans can prevent this problem. The addition of coral sand, calcium carbonate, can be used to supplement calcium in aquaponics with the added benefit of buffering pH.

Sulfur (S) deficiencies are rare, but include general yellowing of the entire foliage in new growth. The amino acids methionine and cysteine both contain sulfur which contributes to some proteins' tertiary structure.

Micronutrients

Iron (Fe) is used in chloroplasts and the electron transport chain. Deficiencies are seen as inter-venous yellowing, followed by the entire foliage turning pale yellow and eventually white with necrotic patches and distorted leaf margins. Iron has to be added as chelated iron, otherwise known as sequestered iron or Fe*EDTA, because iron is apt to precipitate at pH greater than 7. The suggested addition is ½ tsp per large grow-bed whenever deficiencies are suspected; a larger quantity does not harm the system, but can cause discoloration of tanks and pipes. It has been suggested that submerged, magnetic-drive pumps can sequester iron, therefore the use of an external water pump is advantageous.

Boron (B) is used as a sort of molecular catalyst, especially involved in structural polysaccharides and glycoproteins, carbohydrate transport, and regulation of some metabolic pathways in plants. Deficiencies may be seen as incomplete bud development and flower set.

Zinc (Zn) is used by enzymes and also in chlorophyll. Deficiencies may be noticed as poor vigor, stunted growth, and intravenous chlorosis.

Copper (Cu) is used by some enzymes. Deficiencies may include chlorosis and brown or orange leaf tips.

Manganese (Mn) is used to catalyze the splitting of water during photosynthesis, and as such, manganese is important to the entire photosynthesis system. Deficiencies manifest as a dull grey appearance and inter-venous yellowing between veins that remain green, followed by necrosis. Symptoms are similar to iron deficiencies and include chlorosis. Manganese uptake is very poor at pH greater than 8.

Molybdenum (Mo) is used by plants to catalyze reduction-oxidation reactions with different species of nitrogen, and without sufficient molybdenum plants can show symptoms of nitrogen deficiency even though nitrogen is present. Molybdenum is biologically unavailable at pH less than 5.

pH-Dependent Nutrient Availability

The bioavailability of nutrients to the plants depends on the pH of the hydroponic solution [121, 122], which means that the nutrient may be present in the solution but the plant cannot use it. This occurs because pH influences chemical reactions. Chemical interactions such as dissociation and speciation directly affect the uptake of nutrients by plant roots. Sometimes apparent nutrient deficiencies are actually caused by this phenomenon of “nutrient lock-out”, which is specific for each nutrient as seen in Figure 1 [122]. Overwhelmingly, the literature and experts recommend that the best pH for hydroponics is between 5.5 and 7 to prevent nutrient lock-out [123]. However, in aquaponics the ideal pH is a compromise between needs of the plants, the bacteria, and the fish. Nitrifying bacteria are more effective at higher pH, but high pH causes more un-ionized ammonia which is damaging to the fish and plants. To reconcile these environmental requirements, aquaponic systems should be maintained at a pH between 6.5 and 7.

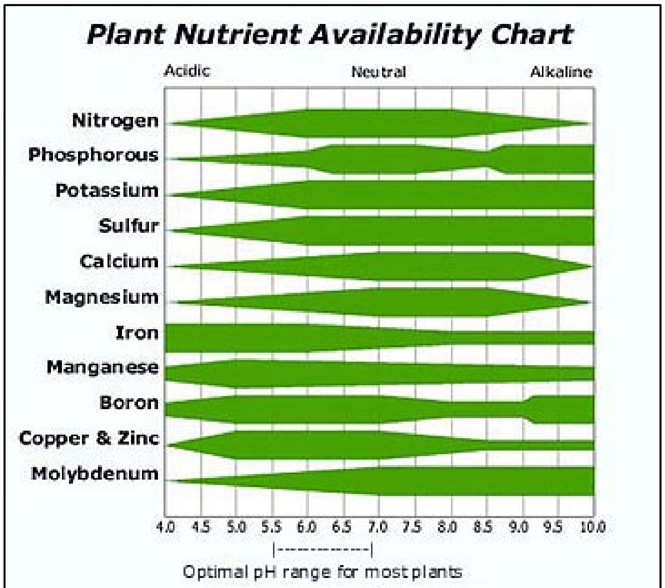

Figure 1: Bioavailability of various nutrients at different pH showing nutrient lock-out, adapted from Fernandez, 2010.

Plants Choice

Some plants are more suited than others to grow in aquaponics. Generally, plants that have fast growth rates, moderate nutrient demand, and at least some tolerance to "wet-feet" are preferred. Tubers and root crops are not commonly grown in aquaponics for the tubers, so potatoes, carrots, beets, and radishes are not suitable options. An interesting exception is turmeric, which grows extremely quickly in particulate beds, develops larger and more attractive rhizomes, and is easier to harvest than in soil culture. Good options include the "hearty-greens", such as kale, chard, collards, and amaranth. Also known generally as spinaches, these are vegetables whose leaves are dark green and traditionally eaten in stir-fry or soup preparations. Celery is included here also. Taro and sweet potatoes can be grown for the greens as well, and in fact, taro leaves are one of the most frequently recommended starter crops. Taro and sweet potato as well as ong choy readily clone in aquaponics making propagation extremely easy. Taro leaves are used in preparations such as lu'au or laulau; these require long cooking times to breakdown the oxalic acid crystals that cause irritating, itchy symptoms in humans unless they are denatured by exposure to prolonged high temperatures. The hearty greens and spinaches are most amenable to particulate grow-bed culture, which provides sufficient support and adequate time to grow.

Other good culinary vegetables that grow well in particulate beds are actually botanical fruits, including okra, tomato, eggplant, chili pepper, cucumbers, and squash. Generally, these crops take longer to grow from seedling to initial harvest, but are then continually harvested for weeks or months. Also, these crops have more standing crop biomass that does best when well-rooted and supported in particulate media. Green onions and chives are a further crop well suited for particulates. Green onions can grow extremely wild in aquaponics and naturally propagate throughout a bed by division and can be harvested every two weeks in optimal conditions.

A suitable choice for deep raft culture are any of the warm weather cabbages, all of which grow well in aquaponics, including kai choi, tatsoi, pak choi, and mitzuna. These plants are well-suited for culture in net-pots in rafts, and also in NFT. These cabbages were observed to take approximately six to ten weeks from seed to harvest. Other excellent options for raft culture are the salad greens, including many types of lettuce and arugula. These crops are extremely well-suited for use in aquaponics, and can grow from seed quickly. However, they are both prone to bolting, where the plant stops vegetative growth and enters a fruiting and flowering stage thereby becoming unpalatably bitter. To prevent this from occurring, salad greens are best grown in cooler seasons or under shade. Water chillers have been shown to prevent bolting by keeping the water temperatures lower, but chillers can add significant cost. Watercress is an exceptional choice for aquaponics; it naturally spreads over slow-moving, nutrient rich water, indeed becoming a pest in some areas. In a deep raft aquaponic design, the rafts can be removed and the watercress grown directly in the water. It is best to provide the watercress with some sort of structure, such as a plastic mesh, or seedling trays, submerged beneath the water to keep it from lying directly on the water.

In addition to vegetables, many of the culinary herbs grow very well. A small aquaponic system can be ideal for a kitchen garden, providing a consistent supply of basil, mint, green onions, parsley, dill, and other herbs. Basil and mint are prolific producers and need to be kept well pruned to prevent woody growth and displeasing flavor. Basil has the secondary advantage of attracting beneficial insects, including nectivores such as ladybugs, lacewings, hoverflies, and parasitoid wasps, as well as pollinators like bees and butterflies. Other good insectary herbs include parsley, dill, fennel, and cilantro. Dry land herbs such as oregano and rosemary grow better in soil, though they can be cloned in aquaponics. Furthermore, many flowers and medicinal plants are suitable for aquaponics, including comfrey, popolo, and feverfew, though no work has shown if and how their efficacy is affected. Citronella and Mosquito Plant are also useful plants, grown to repel mosquitoes.

Temperature, hours of daylight, and other climactic conditions will affect the growth of the plants. The aforementioned recommendations apply to the tropical climate of Hawai'i, and may not be applicable elsewhere. Even so, aquaponics is adaptable and crop choice can be tailored to suit specific conditions. For example, strawberries and trout both prefer lower water temperatures and are a good pair for aquaponics in temperate conditions.

Market and Production Cycling:

Production cycling is a technique that utilizes staggered harvests to meet a consistent market demand. Small, consistent harvests better meet the needs of the consumer, regardless of whether the plant is destined for the family dinner table or the market. It is often desirable to have daily or weekly harvests of lesser amounts of plants rather than a single massive harvest. This is achieved by staggering seed germination and out-planting so that the number of plants entering a system is equal to the number of plants being harvested, resulting in a more constant production [124]. Additionally, a staggered standing crop will ensure the best possible water quality for the fish by pulling a more consistent amount of nutrients out of the water thereby minimizing fluctuations and decreasing the chance of dangerous buildup.

One recommendation is to start seeds in a small seedling tray using a soil-less media such as coconut coir or peat moss. Seedling trays are kept in a germination area that can be environmentally controlled through heat, lights, and irrigation to facilitate early development so that they are ready to be out-planted immediately after a harvest. First, it needs to be determined how long it takes for the crop of choice to grow from seedling to harvest size, known as the time-to-harvest. In the UHM studies, for example, hearty greens and lettuce required a time-to-harvest of eight weeks. If the first two weeks are spent on the germination and seedling table, and not connected to the aquaponics, then the final six weeks are spent in the aquaponic system. Imagine that the goal of the farmer is a single, large, weekly harvest. To determine the amount of area that can be harvested each week, simply divide total area by the time-to-harvest. In this example, one-sixth of the farm would be harvested at any one time. Once harvested, that empty sixth of the farm is immediately replanted with seedlings. At the same time, enough seeds to plant one-sixth of the system need to be started. This is one example of a staggered production system. A wise man once said, "Plant one seed for the birds, one for the bugs, and three for the farmer"; using that advice it is recommended to plant 40-50% more seeds than needed. For crops that grow back in place following harvest, like water cress or green onions, even easier to manage staggered production because there is not re-seeding step. The time between harvests is measured and the whole grow-bed area is divided by that time to delineate sections. It is also helpful to determine the yield of a crop per unit area in order to determine total production. In UHM studies, 40 heads of lettuce or greens could be grown per square meter, but this number depends on specific conditions and crop choice and should be determined for individual farms.

In geographical areas where seasonality is a factor, care should be taken to match the production cycle to the climate. Species of both fish and plant should be chosen to best match the environmental conditions. An annual cycle is observed such that before winter there is a large harvest of both plants and fish. Winter production is very low because fish eat less and plants grow less. Furthermore, outdoor aquaponics need to be winterized to prevent damage caused by freezing. One method is to maintain broodstock and juvenile fish, with the associated hydroponic systems, inside of glasshouses during the winter. A low level of plant production can be maintained throughout the winter especially with the use of grow lights. Following the spring thaw, the juveniles can be moved into the production grow-out stage and the plant production can start anew.

Disease and Pest Management: Plants

Aquaponic systems need an integrated pest management strategy (IPM). IPM consists of four pillars of complimentary techniques used by farmers to mitigate pest damage [125]. First, action thresholds are set to determine the type and abundance of pests that constitute a threat, and further action only occurs if the pests are above that level. A high level of pest species richness with very low abundance is the desired situation, because it supports a healthy beneficial insect population. Though unfortunately common, a program of frequent, broad-spectrum pesticides ensures that no beneficial insects survive, and therefore the pest species can multiply unchecked by nature until the next addition of pesticide. Lotka-Volterra models show that the population of prey has to exist before the population of predators can grow [126], and therefore this technique of spraying broad-spectrum pesticide ensures that the only insect populations are pests, which are able to expand at exponential rates, with increasing pesticide resistance. It is a question of balance, and no action should be taken against the pests until the pre-determined threshold is crossed. Second, consistent monitoring provides early pest detection which, along with correct identification, can inform management decisions in a timely manner. The third pillar of IPM is to use crop diversity. Often disease and pests can be prevented completely by using high crop diversity, resistant varieties and cultivars, and crop rotation. Epidemics and plagues typically occur in monocultures. Finally, if pest abundance crosses the action threshold, a biological, physical, or chemical control needs to be implemented.

Biological and physical controls are the best option for aquaponics [100], because some chemical controls can harm the fish. Areas of the farm should be allocated to trap crops and beneficial insect breeding, which can be as simple as having a weedy hedge line of flowers and trap crops. Beneficial insect populations can be introduced or augmented through purchase from a distributer. Insecticidal soaps and oil extractions of plant's active compounds are often used in organic farming, but oils and surfactants are damaging to fish, especially their gills. There have been reported successes of careful application of Neem oil, sprayed only on the leaves of lettuce in a deep raft grow-bed where no spray was allowed to contact the water. However, extreme care should be used before using any pesticide near an aquaponic system. Another option is to use a physical control of water sprayed to combat some pests. For example, spider mites can destroy crops, especially watercress and tomatoes, but are limited to hot and dry conditions. A fine misting of the crop for one to five minutes every hour can completely prevent this pest. Similarly, sucking insects like aphids and whiteflies and the larvae of many others can simply be washed off with a hose, which kills enough of the larvae to actually make a difference, while leaving the beneficial insect populations intact. Sucking insects that are actively feeding can be killed with this method when their mouthparts are severed by the water pressure. Another trick for nocturnal chewing beetles such as the Japanese beetle is to install solar lights in the garden to disorient the beetle and disrupt the feeding behavior. Terrestrial gastropods, including slugs, African snails and apple snails, can be excluded from grow-beds by installing copper flashing around the perimeter of the support legs which cause an electric effect which will thereby prevent them from crossing it.

Disease Management: Humans - Food Safety

All farming techniques have the potential for contamination by pathogens. Aquaponics has increased safety, namely because the vegetable grow-beds are lifted away from the soil thereby keeping the plants separated from warm-blooded animal excrements that can carry E. coli and other pathogens [127]. It is important to keep vermin away from the system using preventative and control measures, as appropriate. Rats should be trapped rather than poisoned. Fences are useful to keep unwanted wild and domestic animals away. Further precautions can be taken to prevent contaminants from entering the system, namely by ensuring that workers have good hygiene, and by keeping the tanks covered [128]. If water catchment is used sterilization of the water has to be implemented in order to prevent salmonella present in bird guano from entering the system. Food safety is especially important to consider in aquaponics because the water permeates every aspect of the system and any contamination could become systemic.

The primary goal is to prevent any pathogens from getting in the water initially and secondly to prevent the potentially contaminated water from touching any harvestable item. Proper hygiene, including frequent hand washing, and best-management practices can reduce the risk of tainting the food. It is worthwhile to have dedicated harvesting tools such as scissors, clippers, and bins that are used only for harvesting and can be disinfected. These tools must never touch the ground and must be stored in sanitary conditions. Latex gloves should be available and worn appropriately. During harvest and packaging, special attention should be given to plants that are eaten raw, such as salad greens and herbs. Watercress should be harvested in such a way that the crop never touches the water. In fact, the harvestable part of all plants should never touch the water. Besides bacterial pathogens, protozoan parasites should be considered, which usually enter through the feces of gastropods or mammals. Recognizing risks, preventing and controlling the vectors and washing the produce during harvest and packaging and again before preparation greatly decreases the chance of food-borne illnesses.

Conclusion

Aquaponics has received a groundswell of renewed interest in recent decades. The possibilities of increased food production with minimal inputs and minimal environmental degradation are becoming increasingly desirable. Aquaponics is a set of techniques to achieve these goals. Aquaponics is a complex system, much like a living organism, and each part needs to work properly if the whole system is to function. Attention to the design of an aquaponics system, especially in the proportions and installation of critical components, can prevent later difficulties. This article tried to outline some important aspects of aquaponics and offer management solutions to common challenges. Aquaponics is a relatively young field and full of room for ingenuity and research. Hopefully this article provided information through literature review and discussion to guide interested parties in their own aquaponic endeavors.

Techniques of Black Soldier Fly culture: Alternative waste management and feedstuff production

Abstract

Culture of the Black Soldier Fly, *Hermetia illucens,* provides a value-added waste management technique appropriate for processing organic wastes into a valuable feedstuff. Black Soldier Flies, hereafter referred to as BSF, are a cosmopolitan species of dipteran fly of the family Stratiomyidae. BSF larvae are saprophages, consuming organic matter and manure, ultimately becoming a non-pest insect. In BSF culture the larvae feed on otherwise unusable waste, and once developed, a convenient life history trait is exploited by the farmer to collect them. BSF larvae are a valuable feedstuff, with a dry matter composition of 50% protein and 20% fat and are readily accepted by chickens, fish, and swine. Many institutions, villages, farms and communities struggle with the sustainable management of manure and municipal waste, indeed even paying for disposal. BSF culture provides a technique suited for reducing organic wastes, while simultaneously replacing expensive animal feed.

This paper will share important aspects of BSF culture, citing literature and observational data. BSF were cultured at Magoon Agricultural Research Station of the University of Hawaii at Mānoa (UHM), in support of feeding experiments where BSF larvae were used as a replacement of fishmeal in an aquaponic system. The fundamentals of BSF culture are presented in such a way that the reader, farmer, or student would be able to avoid common pitfalls in support of further study of this promising agricultural animal.

BSF as Waste Management Tools

BSF larvae exhibit high levels of various digestive enzymes when compared to other types of insects, supporting the claim that BSF are the most efficient saprophage [129]. In sheer mass, BSF have been shown to drastically reduce the amount of waste. Diener [130] reports a 65-79% reduction in weight of municipal organic waste. Meyers [38] reports a 58% reduction of dairy manure (dry matter basis) with a corresponding

61-70% reduction in phosphorous and 30-50% reduction in nitrogen. These nutrients are stripped from the waste and retained within the body of the BSF, and can be reclaimed later. As an added benefit, BSF reduce the populations of House Fly, *Musca domestica*, and Lesser Fly, *Fannia canicularis*, in chicken houses [34, 131]. Under correct pH and temperature conditions, BSF may deactivate E. coli and Salmonella bacteria; however, the larvae themselves are contaminated with the pathogens [44, 45]. Under laboratory conditions, Popa [132] showed that BSF larvae can complete their lifecycle raised completely on sewage or compost leachate, reducing the volatile organic acids, amines, and alcohols while neutralizing the acidic pH and incorporating nutrients as biomass. Similarly, the greasy residue of biodiesel processing requires proper disposal, but BSF larvae will consume this waste of wastes. Indeed, BSF fed this residue were so high in oil content that they were processed as biodiesel themselves, doubling the overall yield [133]. In summary, BSF larvae eat waste such as manure, carcasses, and food scraps, making these unpleasant wastes safer and easier to manage. Waste passed by BSF, called frass, has reportedly been used directly as a soil amendment, but can be further processed by vermicomposting which results in rich, loamy castings. Besides being a waste management tool, BSF larvae are useful in their own right.

BSF as Feedstuff

In addition to a tool for waste management activities, BSF larvae are an attractive animal feed. High in protein, BSF larvae are an alternative option to the fish meal traditionally used in animal feeds. The larvae have been successfully fed to rainbow trout [40], swine and poultry [37], and catfish [41]. Generalizing these results, animals will eat unprocessed BSF larvae and exhibit growth, though not as much as when fed processed commercial feed. This was the result of UHM studies using BSF as a feed for Hong Kong catfish. More successfully, BSF have been dried, ground, and blended with other ingredients to create more balanced feeds. These feeds had comparable performance to fish meal-based feeds. One negative aspect of BSF as feed is the presence of their chitinous shell, which is completely indigestible to vertebrate animals. If un-ground BSF are used for aquaculture feed the shells can pose a problem by clogging filters and pumps; though UHM studies have shown preliminary success using crustaceans which possess the enzyme to digest chitin to consume the leftover larval exoskeletens. Additionally, methods exist to separate ground BSF into the component parts (protein, fat, minerals, and chitin) but their discussion is outside the scope of this paper.

Collected larvae have been reported in the literature to have excellent nutritional qualities, including, on a dry-matter basis; 40-45% protein, 30-35% fat, 11-15% ash, 4.8-5.1% calcium, and 0.6% phosphorous, as well as beneficial amino acids and minerals [37, 40]. Nutritional analysis of BSF used for the UHM studies were performed by the Agricultural Diagnostic Laboratory Services through the College of Tropical Agriculture and Human Resources. Data from both proximate analysis and detergent analysis presented in Tables 1 and 2, respectively. BSF used for these studies had similar proximate analysis as is reported in the literature. However, BSF contain chitin, not lignin nor cellulose, but the available analyses could not distinguish between these molecules. It is likely that the acid detergent fiber is actually a measure of the chitin.

Nutrient	% of Total
Dry Matter	37.01
	% of Dry Matter
Ash	2.91
Crude Protein	48.96
Crude Fat	26.29
Carbohydrate	21.84
Phosphorous	0.67
Potassium	0.83
Calcium	2.26
Magnesium	0.34
Sodium	0.21
	PPM of Dry Matter
Boron	7
Copper	12
Iron	664
Manganese	103
Zinc	113
Molybdenum	0
Selenium	0

Table 1: Proximate analysis of BSF displayed as a fraction of both dry matter and as-fed basis

Component	% of Total
Neutral Detergent Fiber	60.76
Acid Detergent Fiber	12.49
Lignin	3.39
Cellulose	9.10

Table 2: Van Soest detergent analysis of BSF

The composition of the BSF larvae is influenced by their diet, evidenced in St. Hilaire's report of elevated levels of Omega-3 fatty acids in larva fed fish scraps [134]. Omega-3 fatty acids are essential to fish health and human health [135] The direct linkage between the BSF composition in relation to their feed also suggests that the BSF could be grown on special diets to meet the specific feed requirements of the target animal.

Existing Literature

There have been several notable BSF culture operations. Dr. Sheppard from the University of Georgia pioneered the current culture methods and developed a technical guide for laboratory culture that is indispensible [136]. Sheppard also designed a process of attaching a BSF culture module, consisting of larval harvesting and adult breeding chamber, to existing caged chicken operations. Waste from the hens fell through the floor of the chicken house into an in-ground pit. Adjacent to the waste pit, a captive breeding chamber was attached in such a way that newly hatched larvae could migrate directly to the waste pit [43]. Newton et al. designed a system using a conveyor belt to feed manure into an in-ground cistern inoculated with larvae [137]. Diener designed a system in Costa Rica to process municipal waste in order to investigate the use of BSF as a waste management tool in developing nations [130]. Similarly, Alvarez designed a landfill integrated system to process municipal wastes in Ontario, Canada and examined over-wintering techniques to support the use of BSF [138]. An agriculture research station in Guinea used BSF to process agricultural bio-products and fed the BSF larvae to tilapia, especially welcomed due to the unavailability of standard feedstuff [139]. These papers represent a variety of successful BSF culture operations and demonstrate the feasibility of BSF culture throughout the world.

Life history

Black Soldier Flies have four life stages: Embryo/Egg, Larva, Pupa, and Adult. The cycle starts with an egg hatching as a tiny larva. Once hatched, an average BSF larva is a white, segmented maggot that feeds on moist, decomposing food for approximately six weeks and undergoes five instar stages, or molts. Once it has eaten and developed enough it enters the sixth instar which is about two centimeters long, dark brown, and known as a pre-pupa. Pre-pupae behavior changes from a foraging to a “wandering" phase where pre-pupae attempt crawl away from the feed looking for a safe and dry location to pupate. Pupation takes approximately two weeks, after which time an adult fly emerges. Freshly hatched flies look different than day-old flies. Younger flies have undeveloped wings and are more likely to run than fly, and have slightly larger and softer bodies. Adults fly, living for five to ten days [140] and are entirely engrossed with mating behavior. The adults have no working mouthparts and do not feed; consequently BSF are not attracted to human activities and are not considered a pest insect. Once mated, egg masses of 300-900 eggs are laid near, but not directly in, a food source, preferable in a crack or crevice [141]. Eggs must be protected from desiccation and flooding, and in suitable conditions they hatch in approximately 4 days [34] as a young larvae to complete the cycle. Figure 1 shows photographs of the BSF lifecycle.

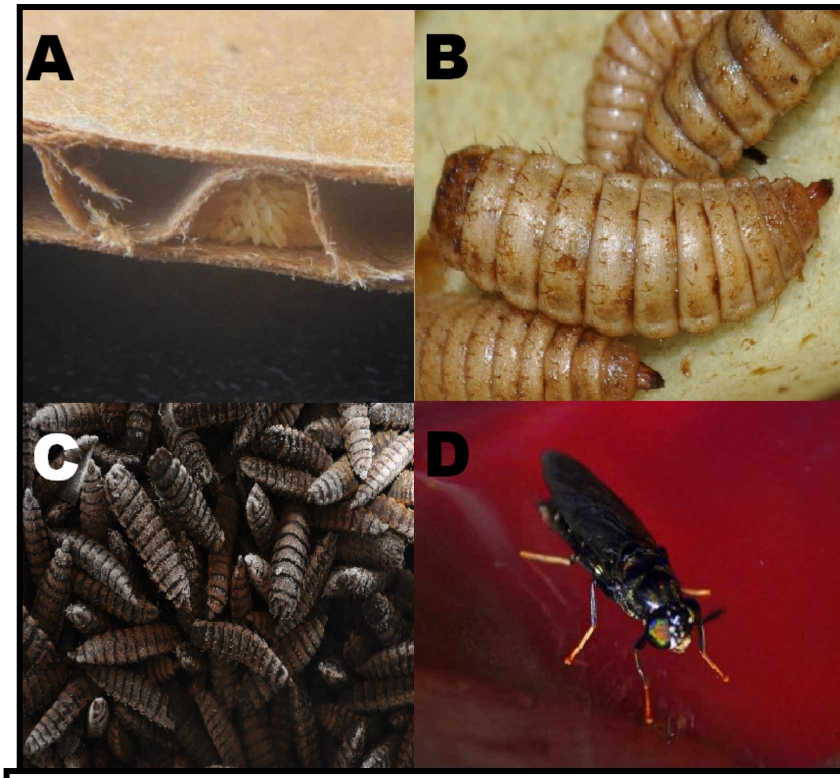

Figure 1: Black Soldier Fly lifecycle: Panel A shows a cluster of eggs, B shows late stage larvae, C shows harvested pre-pupae, and D shows an adult BSF. Images are not to the same scale.

Adult BSF behavior is entirely dedicated to mating. Adults do not eat, but they do require water. Soldier flies are very poor fliers and can be easily caught by hand, and by birds. Suitable conditions for mating behavior need to include ample sunlight and adequate humidity. Suitable habitat in Hawai'i was observed to be a grassy field with a large shade tree. In the study areas, a field of California grass surrounded a shady clearing beneath either Monkeypod or Gunpowder trees. The vegetation is important because BSF exhibit a behavior called lekking. Lekking is a courtship strategy whereby males claim territory on a suitable surface while the females quest for mates by flying past all of the waiting males [142]. Often, BSF adults can be observed near their larval feeding area, waiting on sun warmed grass blades for an opportunity to mate, or resting in the shade during the heat of the day. Mating can occur in the air or on the ground. Soon after copulation the female searches for a suitable place to lay eggs. Larval pheromones and sour scents (like those emanated by fermented grain) are attractive to adult BSF females.

Methods of BSF Larval Culture

A few BSF larvae will be naturally be present in most garbage and compost areas, but methods exist to intensify the operation and allow extensive waste processing and larval harvesting. BSF culture is divided into three parts: the larval grow out where newly hatched larvae feed, a harvesting system to collect prepupae, and an area for adults to mate and lay eggs. BSF culture is scalable, but there appears to be a critical mass; too few larvae result in too few adults to keep the cycling functioning, resulting in low and inconsistent production. Some important aspects of BSF culture are discussed below.

Larval Growout

First and foremost, larvae need to be contained within walls and a roof. The young larvae will not intentionally leave the food source until they are the pre-pupa size, unless the conditions get too hot or there is an exit that they accidentally crawl through. However, if they are moist BSF larvae can stick to any surface (including upside-down horizontal) so integrated containment is essential to prevent masses of escaped maggots. Unfortunately, complete containment is not viable, because the pre-pupae need to exit and be collected. A popular option for small to medium scale BSF production is a larval grow-out chamber called The Biopod™, a trademarked design that consists of a plastic container with a heavy lid seen in Figure 2A. Inside the container is a spiraled ramp. Feed is added to the bucket through the top, the overlapping lid discourages larval crawl-off, and the pre-pupae crawl along the ramp, Figure 2B, where they fall into a collection bucket Figure 2C. This size container can process up to 2 lbs of wet food waste per day and is sufficient for a household. Modifications to this general design are readily available online, varying in size from 5-55 gallons of volume.

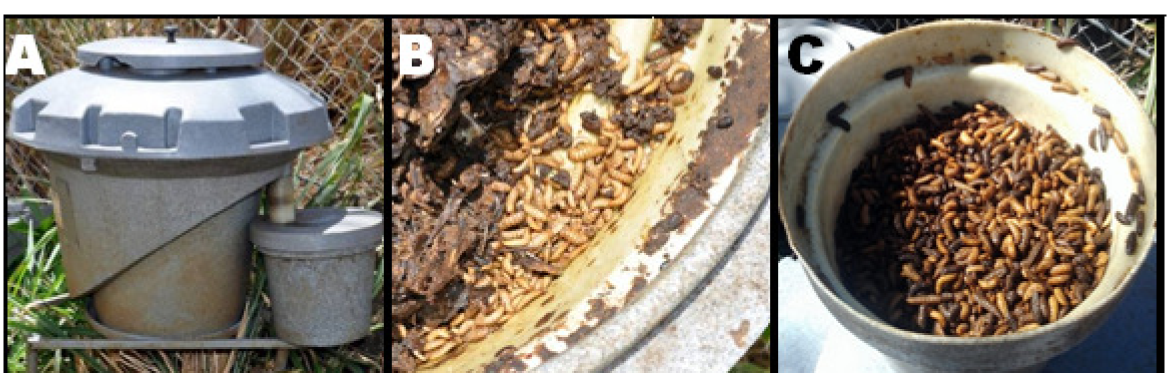

Figure 2: Black Soldier Fly larval grow out chamber, the Biopod™, showing the containment and harvesting components. Panel C contains roughly 3 pounds of BSF larvae

Alternatively, a cistern can be constructed to contain the larvae. The simple design consists of sides made of wood or hollow-form block, entirely lined with polyethylene pond liner. To allow the pre-pupae to escape when ready, a single wall is angled at 30° with a gutter on top. It is recommended that the entire perimeter of the top edge be surrounded with open gutters. These gutters are filled with a desiccating media such as woodchips. This way, larvae or prepupae that exit the feeding chamber are contained within the gutters, for once dried out they are unable to scale the walls of the gutters. From the gutters the BSF are harvested. Harvesting can be done automatically, with a water flushing system, or by hand. This method is preferred for large operations such as those presented by Alvarez in Ontario [138] and Newton and Sheppard in Georgia [143].

Once the BSF are harvested it is worthwhile to rinse them with water. For the UHM experiments, BSF were transferred from the collection bin to a 5-gallon bucket. The bucket was filled with a standard water hose, using pressure to agitate the water. The BSF were strained from the dirty water by pouring them through ½" mesh screening (chicken wire). The water was directed into a standard thermal composting pile. The wire screen with the BSF was then placed atop the same bucket, and the live larvae would migrate through the screen and fall into the bucket. With this double sorting method the ooze and small material was washed out with the water, and any large debris was left behind on the screen as the larvae migrated downwards. Before storage for feed, a final rinse was performed using a fish net under a faucet. BSF were then tumble-dried with paper towels and stored in a plastic container with a tight-fitting lid in the freezer. If BSF were to be stored live, the BSF in the bucket were coated with a thick layer of wood shavings to dry them and prevent them from scaling the sides of the bucket.

Proper environmental conditions are needed for the larval grow out. Though BSF are somewhat robust, production suffers greatly if the conditions are poor. Intense operations need to implement consistent monitoring of temperature, humidity, drainage, oxygenation, and feeding rate. The grow-out chamber needs to be at the right temperature and shaded from intense sunlight. It cannot dry out. It cannot flood. In Hawaii, the ideal location is in the shade of a large canopy tree. Larvae do not need light, so it is possible to bury a cistern in the ground for insulation. Larvae only feed in the aerobic zone and will not burrow deeply into the anoxic zone. Anoxic zones can be caused by poor drainage and over-feeding. Occasional turning of the feed with a pitchfork is sufficient to aerate the bottom layers and make the feed more available. This has the additional benefit of denying anoxic zones to anaerobic bacteria which are responsible for a majority of malodors.

Feed Choice

BSF appear to eat all types of organic wastes. It is easier to list the materials that do not decompose quickly. Fish scales and bones, ti leaves, coconut fiber and mulch are not directly consumed; rather, these materials are eventually composted by associated bacteria. Good feeds include pre-consumer food waste, oils, animal processing offal, and manure. The literature lists chicken manure [43], swine manure [137], municipal waste [130, 138], sewage and compost leachate [132], and processed grease residue [133] as supported feeds. The UHM studies used pre-consumer food waste from the university's main dining hall, and contained primarily starches, grease, meat trimmings, and vegetables. This was supplemented with fish carcasses from various sources.

Feed Rate and Conversion

Diener [36] reports an optimal feeding rate of four kilograms per square meter ($4\ kg*m^{-2}$) of larval growing area. These rates resulted in 145 grams (dry weight) of BSF prepupae per day. Feeding rate needs to be adjusted by the laborers to reflect the larval activity observed within the larval chamber. Consistent underfeeding is far better than occasional over-feeding. All of the feed from the previous day should be processed before more feed is added. Excess feed is the primary cause of houseflies and offensive odors. Ideally the larval grow out chamber would be exceedingly easy to load with waste / larva feed. Loading the wastes into the composter is the most labor intensive part of the duties associated with BSF culture.

Applied Community Structure

BSF and houseflies compete; both of these larvae feed on the same wet waste. Houseflies, unlike BSF, are a serious human pest that can spread disease. The succession ecology, observed during the UHM studies, was that the housefly was the primary colonizer of a new food source. This is supported through forensic entomology research on cadavers to determine post-mortem interval based on BSF oviposition on cadavers [144]. Secondary species include several species of beetles. Only after several weeks will a colony of BSF become visible. The reason for the delay in BSF colonization is that the original, wild BSF have to locate the food source, lay eggs, and time is needed for the eggs develop and hatch. Furthermore, BSF larval development can be slow, taking a full two months to reach the pre-pupa stage. Once the BSF become established, a monoculture develops to the nearly complete exclusion of houseflies and beetles. In fact, it has been demonstrated that the presence of BSF larvae inhibits oviposition of adult houseflies [46]. Rats, mongooses, and lizards will feed on the larvae in the bins if not excluded. Lizards can also eat enough recruiting adults to cause diminished returns. Finally, in Georgia it is reported that 21-32% of pupae can be infected by a parasitoid wasp of the *Trichopria* genus [145].

However, this community can flip between BSF-dominated and housefly-dominated. The concern is that by the time the change is noticed by the manager, it may require several weeks for the BSF to again become dominant. When BSF are thriving, a composter can process a lot of food. However, if the community structure had begun to shift towards housefly-dominated, and there were too few immature BSF larvae to take the place of the harvested BSF pupae, a large influx of feed is too much and will not be consumed. Unless the feed is consumed quickly, foul and anaerobic conditions develop. These anaerobic conditions are always present in the bottom of the bins, but should be as limited as possible. The larvae do not appear to enter the anoxic layers, which remain undisturbed by larval agitation which is what keeps the upper layers aerated. Drainage is difficult in BSF composters, so an undescribed community of bacteria and fungi live within the bottom layer of the composters in a wet and oxygen free environment. This zone is where the demineralization of bones, scales, and lignin occurs. With over-feeding of wet slop, this anoxic zone can extend throughout the composter and cause offensive odors. Also, over-feeding encourages houseflies. Similar difficulties with drainage, larval population crashes, and anaerobic conditions have been seen throughout the current literature [130, 138]. Furthermore, low humidity is fatal [146] to BSF larvae and high temperature can cause larval crawl-off.

Methods of BSF Adult Culture

Insufficient recruitment of wild females causes poor production and undesirable conditions in the larval grow-out and feeding chamber. Poor recruitment may be attributed to ultimate causes of poor scent attractant, inadequate food, weather conditions, more attractive sites nearby, or predation, disease, and parasitism of the adults. Open populations, where the adults are not contained, are difficult to monitor for breeding adults and egg laying, but can be encouraged. Closed populations, where adults are raised in a breeding chamber, are more intensive to manage but ensure consistently high production of eggs and larvae.

Mating

Proper conditions are required to encourage mating and oviposition. Booth and Sheppard [34], report that in Georgia, under natural light, 85% of mating occurs between 12:00 and 17:00 It was reported that females were especially likely to lay eggs on dry surfaces near fresh chicken manure or decomposing chicken feed. There were an average of 998 eggs per egg mass, which took approximately 100 hours to hatch at 24℃ [34]. Survivorship of all life stages is affected by both temperature [147] and humidity [148], where generally warmer and more humid conditions result in shorter egg development time, more surviving larvae, and longer-lived adults. Ideally, the temperature should be between 24-30℃ with at least 60% relative humidity.

Wild female flies will be attracted to compost piles and other food sources, often occurring in standard thermal compost and vermicompost. Certain scents attract BSF better than others. Spoiled grain, with its gentle fermented smell, worked well in the UHM studies. Fermented oats, corn, and brewer’s hops have been shown to attract BSF females. Attractive baits need to be kept very moist, as BSF will not be attracted to dried-out feed. A plastic container can be used within the main composter to hold very moist attractive baits without flooding the rest of the larval chamber. BSF are especially attracted to meat and fish; however, these products will attract unwanted vermin such as rats and mongooses. The very best attractant is an existing colony, whose larvae attract female BSF conspecifics using pheromones. Naturally, a larger BSF operation produces a large, wild breeding population. In a BSF operation with an open population, it is important to have replication and redundancy. With several separate colonies of larvae working, each colony can have a different stage of community structure, but all share the open population of breeding adults. If one colony decreases in production or crashes completely, the wild population of females is still being replaced by the other colonies so that eggs are consistently being laid throughout the operation. However, recruitment of wild females is a recurring problem in BSF operations in Hawai'i. A noticeable warning sign is when an inspection of the larval grow-out reveals no young larvae in a colony, meaning that females have not been returning to lay eggs. To remedy this common problem, the colony needs to be actively managed, ensuring all of the environmental conditions are the best possible. More successful, though, is enhancing the open population with a captive breeding program.

Closed Population and Captive Breeding

In agriculture, a closed life-cycle refers to an operation where a small population of breeding adults is retained from the production grow-out and not harvested. These adults are known as the broodstock. Held in ideal conditions, the broodstock are monitored and bred to create offspring for the following production cycle. Captive breeding of BSF can be achieved by retaining a portion of the pupae and allowing them to develop into adults within a mating chamber, which can be any enclosed space. Once hatched, the adults are provided with conditions to promote breeding, which include ample lighting, ample humidity, adequate space to mate, and a surface apt for egg-laying. Artificial lighting is required in wintertime and indoor breeding. Zheng [149] reports success with a 500 watt, 1.35 mol per m^2 per second, quartz-iodine light, which has a visible spectrum while there was no mating success with a rare-earth lamp with ultraviolet spectrum. If the climate is agreeable, the mating chamber can be housed where it receives natural sunlight. Even so, there are documented year-round captive breeding populations in cold climates, such as the one in Ontario, Canada [138].

To increase BSF production in support of the UHM feeding trials, a simple mating chamber was constructed at the Magoon Research Facility in Mānoa. Two aquaria were used, one inverted atop the other to contain the adults, seen in Figure 3A. Screening was used to cover the space where the top aquarium did not cover the lower one, and provided ventilation. The chamber was located in the shade of a large monkeypod tree where the ambient conditions were mild and humid. Mating was observed in the air and on the sides of the glass, Figure 3B. The sunlight levels and humidity within the chamber were not measured. Moist sponges were kept on the floor of the chamber to retain moisture. Sticks and leaves were provided to promote lekking. Pupae were added to a mulch-filled container within the mating chamber. The females lay eggs near a food source, so another container filled with overcooked, watery, instant oatmeal was offered as larval feed. The watery oatmeal fermented within two days and released a pleasantly sour odor. Though most feeds would do, the oatmeal was chosen because fermented, sour odors are attractive to BSF females and yet not especially displeasing to the researcher. Corrugated cardboard squares, ~3cm x 3 cm, were installed next to the oatmeal container as an egg-laying substrate, Figure 3C. Twice weekly, the feed and cardboard squares were replaced with fresh ones, and the larvae-filled feed container was transferred to the production grow out chamber. This simple method was used because it provided ample larvae to keep the grow-out fully stocked while requiring relatively low maintenance.

Figure 3: Black Soldier Fly adult mating chamber (A), showing an egg mass (B) and adult BSF in copula (C)

Conclusion

The culture of Black Soldier Flies can be an useful addition to a midsized farm or homestead. With limited encouragement, these insects can transform all organic waste produced on the land into a valuable feedstuff. With institutions paying to dispose of their organic waste and farmers paying increasing feed costs, Black Soldier Flies can allow an organization to collect income from both ends while helping to protect the environment from over-exploitation of resources and agricultural pollution.

Preliminary study of Black Solider Fly Larvae as Feed for Hong Kong Catfish.

Abstract

Hermetia illucens, Black Soldier Fly Larvae (BSF), were used as a feed for *Clarias fuscus*, the Hong Kong catfish. BSF larvae were minimally processed, only frozen and diced not dehydrated and reformulated, to match real-world, small-scale operations.

In the first trial, 9 catfish were fed an ad libitum diet of 100% BSF (40% dry matter) for 100 days. The reference treatment was fed 100% Skretting Trout feed (SKT). Average weight of the experimental treatment increased from 28.5 g (CI_{95} ± 3.0) to 54.7 g (CI_{95} ± 9.2), a 192% increase. This was a specific growth rate of 0.64 (R^2=68). The average weight of the reference treatment increased from 26.8 g (CI_{95} ± 2.9) to 78.5 g (CI_{95} ± 13.8), which was a 293% increase. The specific growth rate was 1.06 (R^2=88).

In the second trial, 10 catfish were fed a fixed diet of mixed BSF and SKT feed (66% BSF, 34% SKT) for 74 days. The reference treatment was fed 100% SKT. The average weight of the experimental treatment increased from 149.6 g (CI_{95} ± 14.4) to 219.2 g (CI_{95} ± 15.0), an increase of 146% with a specific growth rate of 0.53 (R^2=75.5). The average weight of the reference treatment increased from 150.6 g (CI_{95} ± 16.2) to 221.0 g (CI_{95} ± 28.5), an increase of 146% with a specific growth rate of 0.53 (R^2=59.4). Though the growth rate for both treatments was similar, there was neither significant difference nor equivalence.

Combined, these preliminary data suggest that approximately 2/3rds of standard feed can be replaced with BSF with minimal sacrifice in growth rate. Used alone, BSF can be used as aquaculture feed but the fish growth is much less than standard feeds. That being said, BSF are economical to raise on small to mid-sized scales, and the decreased growth rate may be off-set with savings on feed. More experiments are needed in BSF nutritional analysis, BSF processing, and effects of BSF diet on metabolism, meat quality, and waste, as well as an economic viability.

Introduction

Aquaculture is an increasingly important source of protein world-wide. Human population has increased faster than wild-caught fisheries production, which is unlikely to continue to meet the growing demand for seafood [1]. Aquaculture has the potential to enhance food security, but there are several concerns that need to be addressed in order for aquaculture to be sustainable. One main concern is the supplemental feed that is required in intensive aquaculture systems, traditionally comprised of fish meal and/or fish oil [150]. Fish meal is processed from wild-caught forage fishes, and the concern is that the demand for fish meal from the aquaculture industry does not relieve enough pressure on the wild fish stocks [2, 11, 12]. In addition, the feed is a significant cost of most aquaculture ventures, accounting for up to half of the total cost of raising fish [16]. Furthermore, a locally produced feed could increase the sustainability of aquaculture by supplying feed to farmers without competition with global markets while reducing the costs of shipping. There has been extensive research into alternative aquafeeds, but most fall short of meeting the full requirements of an aquafeed [13, 23, 151]. Some tested feeds include: beniseed and locust bean meals [19], soybean meal [20, 21], sunflower meal [22], meat industry by-products [23, 24], agricultural by-products [25, 26], highly fecund herbivorous fish such as sand smelt [27] or mosquito fish [28], the nitrogen-fixing water fern *Azolla spp*. [29-33], pre-pupae of the Black Soldier Fly, *Hermetia illucens* [34], and bacterial films grown on natural gas [35]. Generalizing these results, vegetable sources can be used to substitute 30-50% of the fish meal in manufactured feed, but after a certain point the growth and health of the fish become compromised. Overall, aquafeed should be sustainably produced, especially economically and environmentally, and the aquaculture product fed this feed must show strong growth.

One possible alternative to traditional aquafeeds is using the Black Soldier Fly Larvae, *Hermetia illucens*, or BSF [152]. BSF are a species of dipteran fly of the family Stratiomyidae with a world-wide distribution whose larvae feed on decaying organic matter and manure [136]. These detritovore larvae are of interest as an alternative aquafeed because they can contain up to 50% protein on a dry-matter basis. Larvae can be easily cultivated on waste products such as institutional food wastes and manure, thereby reprocessing these wastes into a more useful form [43]. BSF larvae are an environmentally responsible waste management system in their own right, preventing organic wastes from becoming pollution [96]. Once processed, the original waste product becomes insect biomass and insect frass; the larvae are a value added product and the frass is much easier to manage than the original waste [130]. Furthermore, convenient life history traits make BSF culture not labor intensive. BSF larvae self-harvest, willingly migrating from the waste pit into a collection chamber. BSF adults are a non-pest species with no working mouthparts, and as such, are not attracted to human activities nor are they a vector of diseases [131]. BSF larvae are therefore sustainable to culture and harvest, thereby meeting the first criteria of an alternative feed. However, an alternative aquafeed needs to be of high quality as a feed, eliciting strong growth and healthy animals.

To further characterize the feasibility of BSF as an aquafeed, a feeding trial was designed to measure the growth rate of the Hong Kong catfish, *Clarias fuscus*, fed on two diets of BSF larvae. These catfish were chosen for the study because they are easy to raise, have relatively fast growth rates, readily accept BSF, were available, and have a local market demand in Hawai'i. This study was designed to provide preliminary data on the growth rate using unprocessed BSF as a feed to support further dietary formulations.

Methods

BSF larvae were cultured on assorted pre-consumer food waste obtained from the University of Hawai'i at Mānoa Food Services. All BSF larvae were collected before the start of the experiment over the course of several weeks. The food waste was not quantified, but generally consisted of rice and pasta starches, fish heads and tails, vegetable trimmings, egg shells and coffee grinds. The larvae were grown in twin Biopods housed at the Magoon Research Facility in Mānoa. An open population of BSF adults was present at the location because of simultaneous composting projects. Additionally, BSF eggs and larvae were supplemented to the Biopods using a small-scale, captive-breeding program. BSF larvae were harvested, washed, sorted, and frozen in an airtight container until use. The stored feed was thoroughly mixed prior to feeding to ensure a random sample. A proximate analysis was performed by the Agricultural Diagnostic Service Laboratory at the University of Hawaiʻi Mānoa in January 2013.

Feeding Trial I was conducted for 100 days, from December 17, 2012 to March 26, 2013. Fish were housed identical, 37 L glass aquaria. Each aquarium contained a small powerhead which pumped water into a mechanical and biological filter. Nine catfish between 20 and 35 grams were selected randomly from an 8-month old cohort that was artificially spawned in March of 2012 for each treatment. The catfish were weighed and measured in length at the beginning, middle, and end of the experiment. Initially, food was withheld for 3 days to encourage the experimental fish to accept the new diet. The experimental diet was entirely BSF larvae, cut into thirds, roughly 3mm x 3mm, and thawed in warm water. The control diet was Skretting Trout Feed (SKT), 4.5 gram size. Fish were offered more food than they ate the previous day. After 20 minutes uneaten feed was removed and counted.

Feeding Trial II was conducted for 74 days, from July 10, 2012 to September 19, 2012. Fish were housed in an outdoor aquaponic system. The system consisted of a sump, grow-bed, and two parallel fish tanks. The 50 gallon sump contained water plants used as mechanical filtrations and the pump, which pumped water to a 100 gallon grow-bed of O'ahu volcanic cinder planted with various vegetables. These cinders were constantly flooded to within 2 inches of the surface. Water draining from the grow-bed was split into twin 35 gallon fish tanks holding the two treatments of fish and finally overflowing the fish tanks' standpipes to return to the sump. Ten catfish between 110 and 186 grams were selected randomly for each treatment from a 17 month old cohort that was spawned in the laboratory in April of 2011. The catfish were weighed and measured in length at the beginning and end of the experiment. Once measured, food was withheld for 5 days to encourage the experimental fish to accept the new diet. The experimental diet consisted of 66% BSF larvae and 34% SKT. The control diet was 100% SKT. The tanks were fed a fixed diet of 1.5% of the fish's total initial body weight per day. On feeding, the water to the tanks was diverted and the fish were observed feeding for 10 minutes, after which time the water flow was restored and the uneaten food was quantified as it was washed out of the fish tanks.

At the end of each trial the fish were reweighed and re-measured. Growth was considered as "live-weight gain" or the difference between the final weight and initial weight. Average weights were compared between initial and final within the treatment to determine if growth occurred using a Student's t-test. Average final weights between treatments were tested for equivalency using confidence intervals. The final average weights were considered equivalent if they were within 5% of each other; the 5% cut-off was determined *a priori* with expert consultation.

The growth rate was calculated as Specific Growth Rate (SGR), which uses the natural log transformation of the initial and final weights to account for the 3-dimensional aspect of growth, as per [103]. Equation 1 shows the SGR equation,

Equation 1: Specific Growth Rate

$$SGR = 100 * \frac{\ln(W_f) - \ln(W_i)}{T}$$

where W_f is the final total weight in grams,
W_i is the initial total weight in grams, and
T is time in days

Feed Conversion Ratio (FCR) was calculated by dividing the total amount of feed (dry matter basis) by the total live-weight gain. FCR is a measure of how much feed is needed for the fish to grow by one unit. Feed Conversion Efficiency (FCE) is the reciprocal of FCR, and indicates how much growth is achieved for one unit of feed. The Condition Index (CI) of the experimental catfish was compared to the CI of 238 catfish sampled between 2010 and 2013 as data collected from other studies. A natural log transformation of both the length and weight was performed to make the relationship linear.

All statistical analysis was performed using MiniTab 14 and were considered significant with an alpha of 0.05.

Results

The proximate analysis of the BSF larvae is presented in Table 1. BSF larvae were 37.01% dry matter, of which was 2.91% ash, 48.96% crude protein, and 26.29% crude fat. Crude fiber was not analyzed and nitrogen-free extract was not calculated. Van-Soest detergent analysis showed 60% of the DM was digestible in a neutral detergent and 12.49% in an acid detergent. Cellulose and lignin were recorded as 9.10% and 3.39%, respectively. BSF also contained detectable quantities of Phosphorous, Potassium, Calcium, Magnesium, Sodium, Boron, Copper, Iron, Manganese, and Zinc, though no Molybdenum nor Selenium were detected.

Table 1: Proximate Analysis of BSF

Nutrient Component	*% of Total*	*% of Dry Matter*
H20	62.99	0.00
Dry Matter	37.01	100.00
Ash	1.08	2.91
Crude Protein	18.12	48.96
Crude Fat	9.73	26.29
Carbohydrate	8.08	21.84
Phosphorous		0.67
Potassium		0.83
Calcium		2.26
Magnesium		0.34
Sodium		0.21
		PPM of Dry Matter
Boron		7
Copper		12
Iron		664
Manganese		103
Zinc		113
Molybdenum		0
Selenium		0

In Trial I, the catfish were measured on Day 0, 50, and 100. Figure 1 shows the mean weight at each time point for both treatments. The catfish fed the experimental diet (BSF) increased from an initial mean weight of 28.5 g (CI_{95} ± 3.0) to a final mean weight of 54.7 g (CI_{95} ± 9.2), an increase of 192%. The final average weight was significantly greater than the initial weight. The catfish from the control treatment (SKT) increased in weight from 26.8 g (CI_{95} ± 2.9) to 78.5 g (CI_{95} ± 13.8), an increase of 293%, and the final weight was significantly greater than the initial weight. Fish fed the BSF diet grew significantly less than those fed the control diet. The difference of the mean final weights between treatments was 23.8 g (CI_{95} ± 15.5).

The specific growth rate was 0.64 (R^2 68%) for the BSF diet and 1.06 (R^2 88%) for the SKT diet. Weights at each time point were natural log transformed and a linear regression was applied in order to determine the R^2 value of the specific growth rate. The regression equations and R^2 values can be seen for the BSF diet and control diet in Figures 2 and 3, respectively.

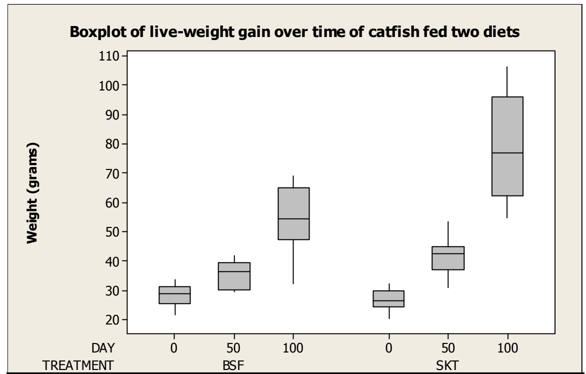

Figure 1: Distribution of fish weight at 3 time points fed 2 diets

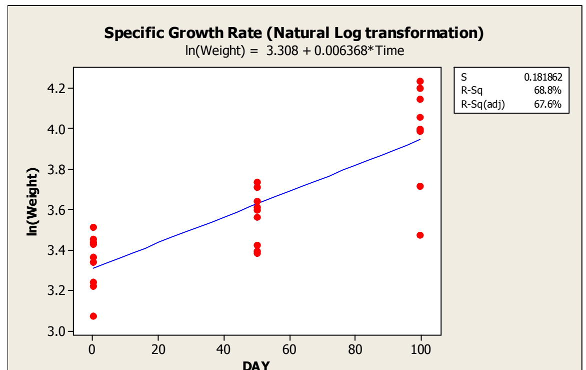

Figure 2: Natural log transformed weights of experimental treatment with linear regression

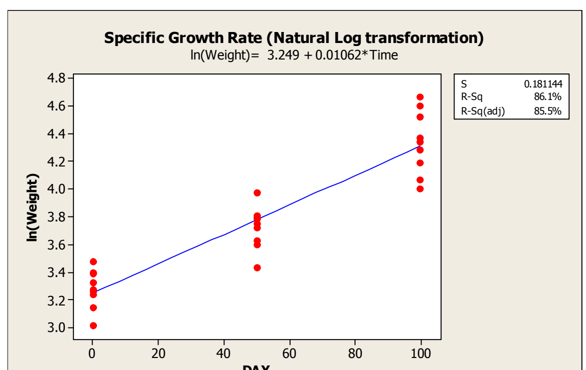

Figure 3: Natural log transformed weights of reference treatment with linear regression

FCR and FCE were calculated for the both treatment diets. However, ratios are supposed to be calculated on a dry-matter feed basis. Only the BSF diet was analyzed for moisture content; SKT feed was not analyzed for DM content and the values are understated. Table 2 summarizes the total weight gain, total feed fed, dry matter conversion, FCR, and FCE:

Table 2: Growth efficiency compared between the two diets, using FCR and FCE

BSF Diet	
Live-Weight Gain	236
Sum of Feed (as-fed)	570
% Dry Matter	37%
Sum of Feed (dry-matter)	211
FCR	0.89
FCE	1.11
SKT Diet	
Live-Weight Gain	466
Sum of Feed (as-fed)	452
% Dry Matter	-
Sum of Feed (dry-matter)	-
FCR	0.97
FCE	1.03

In Trial II the catfish were measured on Day 0 and 72. Figure 4 shows the box-plot of average weights of both treatments at each time point. The catfish fed the experimental treatment increased in mean weight from 149.6 g (CI_{95} ± 14.4) to 219.2 g (CI_{95} ± 15.0), a significant increase of 146%. The catfish fed the control diet increased in mean weight from 150.6 (CI_{95} ± 16.2) to 221.0 (CI_{95} ± 28.5), a significant increase of 146%.

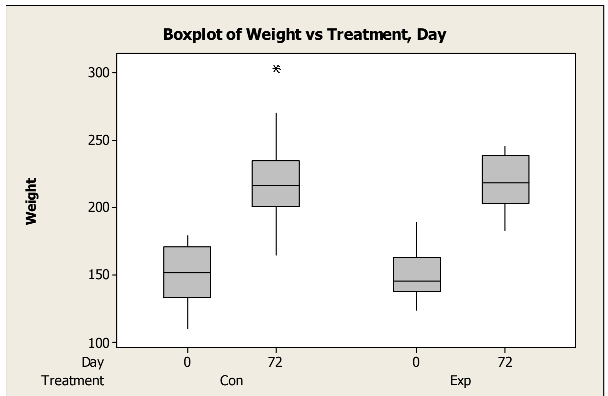

Figure 4: Boxplot of initial and final weights of two groups of catfish fed different diets

Figure 5 shows the 95% confidence intervals of the final average weight of the two treatments. It was determined, *a priori*, that the means would be considered equivalent if the difference between the means was less than 10 grams, which is 5% of the final weights. The mean final weights were very similar between the treatments, 219.2 g and 221.0 g. However, the 95% confidence intervals of the both treatments were greater than this pre-determined allowable difference, and thus equivalency was rejected at an alpha = 0.05.

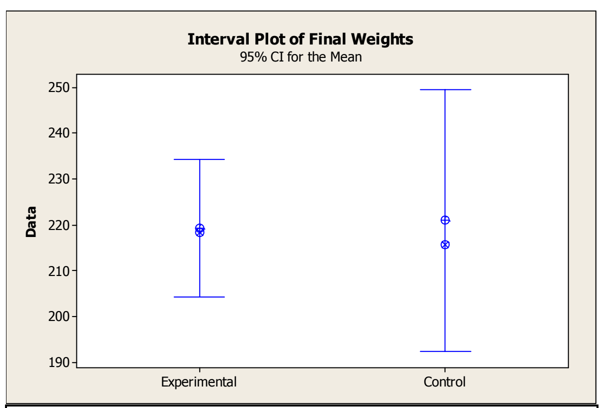

Figure 5: Confidence intervals of final weights of two groups of catfish fed different diets

The specific growth rate of both treatments was calculated be 0.53. Figures 6 and 7 show the equation for the specific growth rate for each treatment with the corresponding R^2 value.

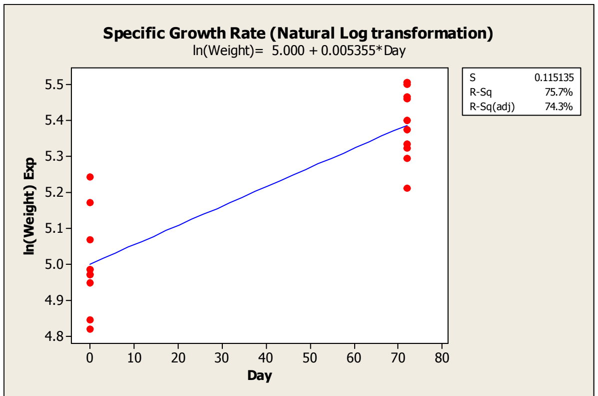

Figure 6: Specific growth rate of experimental treatment catfish

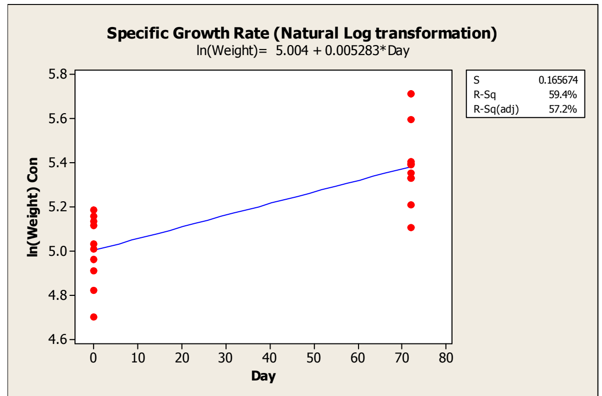

Figure 7: Specific growth rate of reference treatment catfish

None of the fish in the treatments from either trial were particularly skinny or fat for their length. Figure 8 shows the condition index (Weight/Length) from all catfish measured since 2010. There are few outliers, in general. All of the experimental fish were less than 25 cm and are included in this figure though no dedicated statistics were performed to determine if the experimental treatments fit the regression as well as controls. Figure 9 shows a fitted line plot of the natural log transformed weight and length. A linear regression shows an R^2 value greater than 97% indicating a strong correlation. The equation to predict the weight based on the length is: $Weight = 4.9058*e^{0.1326*Length}$

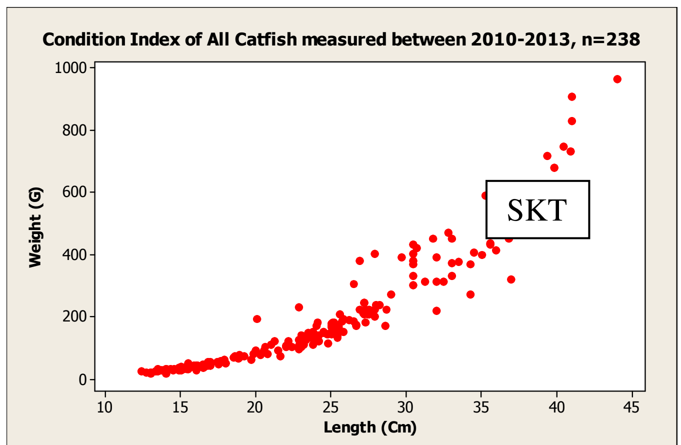

Figure 8: Condition Index of all catfish measured during these studies

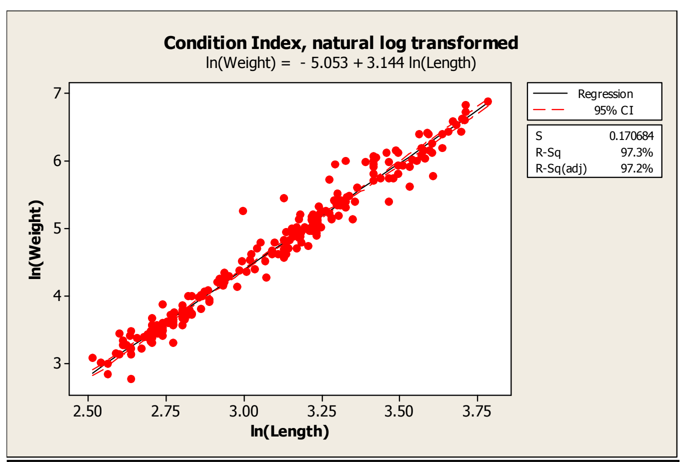

Figure 9: Regression line of transformed condition index of all catfish measured during these studies.

Discussion

This preliminary trial showed that catfish will eat BSF Larvae. All of the fish survived, suggesting that there were no significant anti-nutritional factors in the BSF. Temporally, Trail II was conducted before Trial I. In Trial II, both treatments showed remarkably similar growth. However, the trial was too short, and it could be suggested that the growth between treatments would be different over time. Generally, aquaculture feeding trials should be long enough for the fish to double in size. These fish only increased, on average, by 47%, or half as long as would have been needed. This trial could have been continued, but the logistics of an additional 11 weeks proved insurmountable. Additionally, these fish stopped feeding for over 2 weeks after handling on day 72. The handling effects could have been lessened by conditioning the fish with repeated handling before the beginning of the experiment using reinforcement. Furthermore, the outdoor aquaponic set up had a serious disadvantage in that it was difficult to observe and quantify feeding because the sides of the tank were coated with dark algae that made it difficult to see the BSF larvae. Some of the BSF larvae sank, and it was difficult to measure the amount that sank and remained un-eaten, whereas floating feed was easy to quantify.

One of the interesting results was the high of variability in the control tank. One short, fat fish is clearly visible in Figure 4. These fish were just entering sexual maturity, and two fish extruded eggs during handling. It is possible that this fat fish had put more energy into gonad development rather than length. Also common in fishes, depensatory growth is when dominant fish consume more feed than subordinate fish resulting in high variability of the average size [153]. This high variability contributed to the fact that we had to reject our statistic for both difference (t-test) and equivalency.

Using lessons from Trial II, it was decided to repeat the experiment with three changes. First, smaller fish were used so that they could double in size in a reasonable time. Second, Trial I was conducted inside the laboratory in clear glass aquaria so that feeding could be clearly observed. Finally, the experimental treatment was 100% BSF at an ad libitum feeding regime to attempt to find the baseline growth rates.

The treatment fish grew in Trial I. The final average weights were greater than the initial weights, answering an important question of this study. Catfish survived and grew by eating 100% unprocessed BSF larvae. However, the growth rate was less than that observed by the control. An important factor in that was that unprocessed BSF are primarily water; 60% of the larvae's weight was non-nutritive though it fills the stomach and limits further ingestion. Though the experimental fish ate more total feed by weight, on a dry-matter basis they ate far less than the control, partially explaining the difference in growth. On a dry-matter basis, however, the feed conversion ratio and conversion efficiency were encouraging.

Temperature is known to affect the growth rate of fish. Anderson [154], demonstrated that growth was almost double in juvenile catfish raised at 25°C than 20°C. This may explain the low feeding rate of both treatments in Trial I. Furthermore, fish grow faster if they are fed to satiation multiple time per day instead of as on time per in these studies [153].

However, the experimental feed was free (minus labor) while the control fed is ~1$/lb. So even though the fish grow slower on BSF, the decreased cost of production makes BSF an attractive option. Furthermore, in aquaponics the plant production is often more profitable than the fish production so further studies will investigate the water quality of culture water where BSF were used as feed.

Overall, these results support that BSF can be used as an aquafeed as reported in the literature with certain caveats. Sealey reports that Rainbow Trout exhibited slow, but positive growth on 100% BSF diets, and grew as fast on diets with up to 50% of fish meal replaced with BSF meal [155]. Sheppard reports that Channel Catfish showed identical growth fed up to 30% BSF [43]. St-Hilaire reports that Rainbow trout showed no difference in growth or FCR on diets up to 15% BSF meal, though Omega-3 fatty acids were lower [40]. Processing the BSF in to dehydrated, ground meal increases the quality of the feed, especially if formulated with other feed amendments. Further research will be conducted on low-technology methods of processing that do not unduly increase the labor costs.

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