Supplemental Duckweed in the Diet of Blue Tilapia (*Oreochromis aureus*), and the Potential for Implementation at Allegheny College

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	4/24/15	Eric Pallant	4/23/15
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The aquaponics system at Allegheny College is designed to demonstrate a sustainable means of growing food. However, the most unsustainable input is the fishmeal pellets that the fish are currently fed. A proposed solution to the impact of the pellets is to supplement them with a vegetable-based protein source. This study investigated the effects of fresh duckweed as a dietary supplement for Blue tilapia, as well as whether a difference exists between the effects of fresh and dried duckweed. It was hypothesized that supplements up to 30% would produce similar results to those of all-pellet diets, and that fresh and dried duckweed would yield similar results. For Trial 1, treatments of 0%, 30%, and 50% duckweed supplements were used to compare specific growth rates (SGR) and feed conversion ratios (FCR) of tilapia in tanks, with three replicates for each treatment. In Trial 2, supplements of 30% fresh and 30% dry duckweed were compared using the same replicate methods as Trial 1. Results of Trial 1 indicated a significant difference among treatments for both SGR and FCR. Specific growth rate values decreased and FCR values increased with increasing levels of supplement. Results from Trial 2 indicated no significant difference between using fresh or dry duckweed. Based on the results of this study, duckweed supplementation could be viable in small quantities for tilapia. At Allegheny College, where fast growth is not vital to the system, it is recommended that dry duckweed be supplemented in small quantities to decrease the amount of pellet feed consumed.

INTRODUCTION

Approximately one billion people globally depend on fish as their main source of animal protein, and around 17% of all animal protein consumed globally in 2009 came from fish (Engelhaupt, 2007; FAO, 2012). As natural fish stocks decline, the need for aquaculture to produce more fish for food and other uses grows (Cahu, Salen, & de Lorgeril, 2004). Worm et al. (2006) predicts that all wild fish stocks will collapse by the year 2048, which would not only have serious impacts on human food supply, but also on oceanic ecosystems that provide vital services.

Aquaculture, the process of farming aquatic organisms for human consumption and other uses, accounts for nearly half of all fish produced as food around the world (Food and Agriculture Organization [FAO], 2014). On average globally in 2012, almost 10 kg of fish per person was supplied through aquaculture. This is an important statistic as 60% of assessed wild fish stocks in 2011 were fully exploited, meaning they cannot sustainably be exploited any further for food (FAO, 2014). The aquaculture industry is capable of producing almost 600 different species of fish worldwide, nearly triple the number in 2000, of which the most widespread is tilapia, being raised in 140 countries around the world (FAO, 2014; Naylor et al., 2000). A global shift from capture fisheries to aquaculture would also have lasting benefits for the atmospheric environment, as current commercial fisheries rely heavily on fossil fuels that have damaging environmental impacts (Pauly et al., 2003).

While aquaculture can have positive effects on the environment, such as decreased impacts on wild fish stocks, it can also have detrimental impacts on both the environment and the fish themselves if not done properly. Fish excrete large amounts of nitrogenous waste, mostly in the form of ammonia (El-Shafai, El-Gohary, Nasr, van der Steen, & Gijzen, 2004). This nitrogenous waste and waste in the form of suspended solids are not only toxic to the fish themselves, but can also end up in local waterways and cause problems such as eutrophication and oxygen depletion (Aubin, Papatryphon, Van der Werf, Petit, & Morvan, 2006; Cole et al., 2009). Around 2.2 pounds of toxic ammonia can be produced for every 100 pounds of fish feed put into an aquaculture system, making this an important issue (Masser, Rakocy, & Losordo, 1999).

A possible solution to the issue of excess nitrogenous waste in local waterways is the use of a recirculating system to prevent ammonia and particulate matter buildup (Masser et al., 1999; Rakocy, Masser, & Losordo 2006). Recirculating systems are complex, but if done correctly can yield high volumes of fish biomass in a small area (Masser et al., 1999). These systems involve cleaning fish wastewater to allow it to be recycled back into the system. The process involves filtering solid wastes, converting ammonia into nitrates, and aerating the water before reusing it in the system (Losordo, Masser, & Rakocy, 1998). While all recirculating systems involve these steps, not all systems are the same, and some types of recirculating systems include more complex steps in addition to the basics. An example of a more complex method is an aquaponics system.

Aquaponics is the combination of hydroponics and aquaculture, in which crops are grown directly in water without the use of soil and fish are farmed for consumption. An aquaponics system utilizes beneficial bacteria, which convert ammonia first into nitrites, and then into nitrates. At moderate levels, nitrates are both safe for fish and useful to plants that take up water from the system, as nutrients are more easily absorbed by plants directly from water. This cyclical system allows more fish to be farmed in a given volume of water and also allows plants such as lettuce or herbs to be grown easily and without soil (Goodman, 2011; National Sustainable Agriculture Information Service, 2010; Rakocy et al., 2006).

While aquaponics systems have the capacity to be very sustainable due to their limitation of nutrient waste, there are factors that can limit sustainability. These include inputs such as light, extra nutrients, water, heat, and fish food (Rakocy et al., 2006). If these inputs are not produced sustainably, the sustainability of the system as a whole decreases. An example is electricity produced from a coal power plant instead of renewable sources of energy.

A way to combat this issue is to ensure that all inputs are produced with minimal impact on the environment. Fish food is often the most environmentally and economically unsustainable input, and therefore its incorporation into aquaculture systems has much room for improvement El-Sayed, 2004; Jackson, Capper, & Matty, 1982; Khan, Siddique, & Zamal, 2013; Naylor et al., 2000; Shiau, Lin, Yu, Lin, & Kwok, 1990; Tacon, Hasan, & Subasinghe, 2006). A life-cycle assessment of salmon farming in four different countries done by Pelletier et al. (2009) showed that feed in these systems accounted for the majority of cumulative energy use, biotic resource use, greenhouse gas emissions, and acidifying emissions. In addition to being environmentally

unsustainable, the protein component of commercial fish feeds is the most expensive and also one of the largest contributors to fish growth, one of the most important aspects of fish farming (Chou & Shiau, 1996; Gaber, 2002; Hanley, 1991). Fishmeal, sometimes from wild fish stocks, is often the primary source of protein and nutrients to fish in aquaculture, which means finding a cheaper and more sustainable source of protein is vital to increasing the sustainability of an aquaponics system as well (DeLong, Losordo, & Rakocy, 2009; Tacon & Metian, 2008).

The aquaponics system in the Environmental Science department at Allegheny College produces both tilapia and romaine lettuce. Currently, tilapia in the system are fed a complete diet of fishmeal pellets, which are both expensive for the department and the most unsustainable aspect of the system as a whole. Because of its tendency to grow quickly and easily and success in previous studies, duckweed could be a potential supplement to the diet of tilapia at Allegheny College, replacing a significant portion of their fishmeal consumption.

The goal of this study was to investigate whether duckweed supplements could be viable at Allegheny College. For this project, duckweed was supplemented into the diets of Blue tilapia (*O. aureus*) to determine whether growth rates were affected by levels of supplementation. Another experiment determined whether there is a difference between the effects of fresh and air-dried duckweed on growth. Air-dried duckweed, if nutritionally feasible, could benefit the department by allowing food to be stored for extended periods of time and be used as dry feed. It was hypothesized that levels of supplemental duckweed up to 30% could provide enough nutrition to maintain good growth rates in the aquaponics system at the College, and that dried duckweed could produce similar results to those of fresh duckweed.

BACKGROUND

Tilapia are one of the most popular fish in aquaculture and aquaponics systems due to their ability to tolerate high fish densities in artificial systems and their resistance to bacterial infections (Ayaad & Hassouna, 2003; DeLong et al., 2009; Winfree & Stickney, 1981). They are also known for their ability to grow on supplemental vegetable-based sources of protein instead of a protein diet consisting entirely of fishmeal (DeLong et al., 2009; Köprücü & Özdemir, 2005). The protein requirement for the diets of tilapia fingerlings is around 35-40%, making it crucial for growth at this early life stage (FAO, 2015). Most commercial fishmeal feeds meet or surpass these requirements, making them ideal for young tilapia. Because fishmeal pellets are

designed specifically to meet the protein requirements of tilapia and other finfish, the protein provided by this diet is unlikely to be fully replaceable by another source. However, dietary supplements of other protein sources could potentially lessen the impact from commercially produced fishmeal feeds.

Numerous studies have investigated different food items as a replacement or supplement for diets of tilapia. Farm-raised tilapia are most often fed a diet of fishmeal pellets and are one of the largest consumers of industrial fish pellets in aquaculture (Tacon & Metian, 2008). Some high-protein, natural plant-based materials such rapeseed meal, moringa leaves, rice wine residual, duckweed, *Leucaena* leaf meal, cassava leaf meal, barley, and soybean meal have all been proposed as potential supplements to fishmeal as food for tilapia to lessen the environmental impact of the pellets (Belal, 1999; Davies, McConnell, & Bateson, 1990; Hassan & Edwards, 1992; Ng & Wee, 1988; Nyirenda, Mwabumba, Kaunda, & Sales, 2000; Richter, Siddhuraju, & Becker, 2003; Vechklang, Boonanuntanasarn, Ponchunchoovong, Pirarat, & Wanapu, 2011; Wee & Shu, 1989; Wee & Wang, 1987;). The majority of these supplements appeared to be feasible in small quantities, but few were considered viable as substantial supplements. Duckweed produced some of the best results, showing comparable growth to fishmeal up to 30% supplement.

Wee and Wang (1987) investigated the possibility of using *Leucaena* leaf meal as an ingredient in fish feed for tilapia. They tested levels of 25%, 50%, and 100% protein from soaked, sundried, and commercially produced leaf meal over 10 weeks, and found a decrease in both growth and feed utilization with an increase in *Leucaena* leaf meal. The soaked leaf meal did produce better results than either sundried or commercially produced meal, but overall it was determined that *Leucaena* leaf meal would most likely not be an effective supplement in the diets of tilapia.

Two years later, Wee and Shu (1989) conducted a similar experiment, which instead included boiled soybean in pelleted feed. While large amounts of unboiled soybean inhibited growth in tilapia due to high amounts of trypsin inhibitor, including boiled soybean as the sole plant-based protein source did produce a relatively high growth rate. These results suggest that that boiled soybean could be effective in supplementing tilapia feed, and also that the simple percentage of a supplement may be less important than the role the supplement plays in diet. Another soybean meal experiment was conducted by Nyirenda et al. (2000), which showed

slightly different results. In this study, trials received all animal protein, all soybean meal, or no protein at all. This differed from other experiments in that there were not increasing levels of supplement. There was no significant difference between growth rates across the trials, suggesting that all animal protein can be successfully replaced by soybean meal protein in tilapia diets. These results differed from those of Wee and Shu (1989), possibly because two different species of tilapia were used. Nyirenda et al. (2000) also did not specify whether boiled or unboiled soybean meal was used.

Results of a study conducted by Ng and Wee (1988) showed a similar pattern as those for *Leucaena* leaf meal using cassava leaf meal in supplements of 20%, 40%, 60%, and 100% over 10 weeks. A trial was done for both soaked-then-sundried cassava and sundried-only cassava. Growth rate of tilapia decreased as cassava percentage increased, both for soaked-then-sundried and sundried only cassava. It was concluded, however, that cassava leaf meal could be a viable partial supplement in feed pellets when paired with traditional protein sources.

Davies et al. (1990) investigated rapeseed meal as a 15-60% supplement over 56 days. As diet composition changed to include more rapeseed, feed intake and specific growth rate both decreased. A supplement of 15% rapeseed meal was suggested as a means of improving the feed input while still mostly maintaining growth in the fish. These results are similar to those of Richter et al. (2003), who used moringa leaves instead of rapeseed for seven weeks. They tested levels of 10%, 20%, and 30% moringa leaf, and determined that 10% moringa leaf supplement is the maximum that should be used in tilapia feed without compromising growth.

Belal (1999) used barley as a dietary supplement for corn in tilapia feed. There were four increments in this study: 0%, 15%, 30%, and 51% barley. The trial lasted nine weeks. It was found that the 15% and 30% supplements showed the highest growth rate, while the 0% and 51% treatments showed a significant reduction in growth. This study suggests that barley can be included as a substantial supplement to normal tilapia feed, but should not be considered a replacement to traditional food sources without a reduction in growth.

Duckweed is a common floating pond plant, often considered undesirable by pond owners because of its tendency to create thick mats on the surface of the water. In recent years, however, duckweed has gained popularity in the world of aquaculture and aquaponics due to its fast growth rate, easiness to grow, and high protein content (Edwards, Hassan, Chao, & Pacharaprakiti, 1992; Journey, Skillicorn, & Spira, 1993). Of all common plant-based protein

sources, duckweed is often considered the closest nutritionally to animal-based protein sources. It is a known foodstuff for multiple species of fish and can provide complete nutritional requirements for many of those (Journey et al., 1993). While duckweed can provide nutritional requirements for fish in household ponds, growth is most important for commercial fisheries, which may only be able to utilize supplemental duckweed in order to maintain normal growth rates.

Duckweed as a dietary supplement for several species of tilapia has been the focus of several studies. Gaigher, Porath, and Granoth (1984) did not conduct percentage treatments as many other supplement studies have done. This study focused simply on the comparison between duckweed as the sole food and duckweed when combined with fish pellets over an 89-day period for hybridized tilapia, *Oreochromis niloticus* × *O. aureus*. Duckweed as the sole feed produced low growth rates, but also produced a good feed conversion ratio, which can also be important for aquaponics systems as food is used efficiently. The diet of mixed duckweed and pellets produced results similar to those of a pellet-only diet, but with a higher feed conversion ratio, which represents the number of units of food mass required to increase body mass by one unit. These results are promising because although duckweed as the sole food source would not be effective at producing high growth rates, supplemental duckweed could be comparable to traditional pellet-only diets in commercial systems.

Hassan and Edwards (1991) compared two different types of fresh duckweed (*Lemna perpusilla* and *Spirodela polyrrhiza*) to see which performed better over a 70-day period. *Lemna perpusilla* yielded higher growth rates and better feed utilization efficiency. One possible explanation for these results is that *Spirodela* duckweed generally has larger fronds and high crude-fiber content. *Lemna perpusilla*, on the other hand, performed well, and was recommended as a possible alternative to animal proteins in the diets of tilapia. It was also determined that 1-6% of the total fish body weight is the ideal feeding rate of duckweed.

Fasakin, Balogun, and Fasuru (1999) conducted experiments with duckweed similar to those of other supplements. Over 56 days, they tested 5%, 10%, 20%, 30%, and 100% levels of solar-dried duckweed supplements in tilapia diets to see which was the optimum amount. Results suggested that supplements of up to 30% duckweed could be viable in systems currently using fishmeal. Mbagwu and Adeniji (1988) also investigated the economic practicality of using dried duckweed as feed for both poultry and fish, and found that harvesting and drying the weeds were

economically feasible throughout the year. Yields were high enough to supply to both poultry and fish.

Based on these studies, it seems possible that fishmeal can be supplemented with a plant-based food source such as duckweed, although methods for determining the viability of supplements varied, possibly leading to varied results. In high quantities, most supplements, including duckweed, appear to lower growth rate. However, in medium to low amounts, supplemental plants and especially duckweed appear to be feasible both economically and for growth.

Because duckweed as a supplement has been widely studied, few gaps exist in the research. However, while these studies focused on either dried duckweed or fresh duckweed, none investigated a comparison of the two. It would be beneficial to have both fresh and dried duckweed in the same study to compare growth rates of the two, instead of solely comparing duckweed to traditional diets. This information would be valuable because dried duckweed has the potential to be stored longer than fresh duckweed, and if both types produced similar results, it would be possible to efficiently manage duckweed harvesting and storage without compromising growth in tilapia.

This experiment aims to both investigate the effects of fresh duckweed supplements in comparison to fishmeal feed and determine whether a difference in growth results from supplementing fresh and dried duckweed into the diets of Blue tilapia. It was hypothesized that supplements of fresh duckweed up to 30% would produce growth rates similar to those of all-pellet diets, and that fresh duckweed and air-dried duckweed would produce similar growth rates.

MATERIALS AND METHODS

Methods were based on those of Justin Gaudi (2011), who performed a similar experiment with germinated barley. Two trials were conducted, one in the fall of 2014 and one in the spring of 2015, to test each part of the hypothesis. The first trial investigated varying amounts of supplemental duckweed to determine the amount of supplement that could be added without drastically compromising fish growth. In the second trial, this supplement was reinvestigated by

comparing wet and air-dried duckweed to determine whether there was a difference in tilapia growth between the two.

Trial 1

Trial 1 lasted a total of 5 weeks (35 days). Mixed-sex Blue tilapia (*Oreochromis aureus*) were used for the investigation, as these tilapia are commonly raised in aquaculture systems. A mixed-sex group was used because it is difficult to determine the sex of tilapia before they reach 25 grams. Tanks were 10 gallons each, and each contained gravel and a Lee's Economy Corner Filter with oxygen supplied. Each tank contained 10 randomly selected tilapia. Water temperature was kept at 27 degrees C using a heater.

There were three treatments: control, 30%, and 50%, with each percentage representing the mass of duckweed supplemented into the diets. The remaining portion of each diet contained Zeigler Silver® floating fishmeal pellets, and the control received 100% pellets. Each treatment had three replicates, for a total of nine aquariums.

The masses of the fish were recorded initially and at the end of the trials, as well as weekly throughout each trial. Both total mass and average mass for each tank were recorded. Total mass was used to measure food amounts, and average mass was used in case of fish mortality to allow comparison of growth between tanks.

Duckweed (*Lemna minor*) was grown in water-filled bins in a greenhouse by starting with an initial amount and allowing it to spread on its own. Tilapia wastewater was added to bins to promote faster plant growth. After harvesting, duckweed was allowed to air dry for 24 hours prior to feeding to allow excess water to evaporate before massing. Any duckweed not used immediately after the 24-hour period was stored in an airtight container to prevent any further drying and allow for equal mass comparison.

The total mass of fish in each tank was used to determine the total amount of food given to each tank per day. Each tank received 5% of its total mass in food each day, and supplement levels were calculated for each treatment from the 5% mass of each tank. The total daily diet was halved and given to fish in two daily feedings, each 8 hours apart. Early in the trial, fishmeal pellets were ground into smaller pieces due to the small size of fish, and duckweed was added to tanks before pellets to allow the tilapia to learn to eat it. Diets were adjusted weekly with each weighing to reflect the new total weight of each tank.

Trial 2

Trial 2 lasted a total of 4 weeks (28 days). Two treatments of 30% duckweed supplement were compared: one of fresh duckweed, which acted as a control, and one of air-dried duckweed. Each treatment had three replicates for a total of six aquariums.

Duckweed was grown using the same methods as Trial 1. After harvesting, duckweed was divided into two groups. One was air-dried for 24 hours and stored in an airtight container to maintain freshness, as was done in Trial 1. The second set of duckweed was air-dried for an additional 48 hours, until fully dry. Duckweed masses for both treatments represent mass after the 24-hour drying period, to allow for accurate comparison. The feeding and massing procedures were the same as those of Trial 1.

Statistical Analyses

Specific growth rate (SGR) and feed conversion ratio (FCR) were calculated for each tank using the average mass of fish. The SGR represents the rate of change of the logarithm of weight and time, and indicates the change in cell mass per unit of time (Elliot & Hurley, 1995). It was calculated using Equation 1, in which W_t represents the final mass of fish after the given time period, W_0 represents the initial mass of fish at the start of the trial, and t represents the time during which the trial was conducted (Elliot & Hurley, 1995). In this case, t was measured in days, and therefore SGR represents the daily increase in mass throughout the trial.

Eq. 1 $$SGR = 100\{\ln(W_t / W_0)\} / t$$

The FCR represents the ratio between food consumption and weight gain in the tilapia, indicating the number of units of feed mass required to increase body mass by one unit. The FCR was calculated using Equation 2, in which F represents the average mass of food consumed by each fish per day, t represents the number of days during which the trial was conducted, W_t represents the final mass of fish after the given time period, and W_0 represents the initial mass of fish at the start of the trial (Chou & Shiau, 1996).

Eq. 2 $$FCR = (Ft) / W_t - W_0$$

It is important to note that the FCR technically requires a value for feed consumed each day, while this experiment used the value of feed given to fish each day, as uneaten food was not quantified and taken into account. However, since young tilapia are known to eat up to 5% or more of their body weight daily, it can be assumed that the difference between food presented and food consumed was minimal (Gaber, 2002; Riche & Garling, 2003).

A one-way analysis of variance (ANOVA) was used to compare SGR and FCR values among treatments in Trial 1. This was the chosen statistical test because three independent means were being compared to detect differences between these values. The null hypothesis for this test is that no difference exists among treatments, and significant results indicate that a difference exists between two or more treatments. The ANOVA was followed by a Tukey HSD post hoc test, using a 5% significance level. The results from a Tukey HSD test indicate where the differences lie between treatments, making interpretation of the results more meaningful and accurate.

A two-sample non-directional t-test was conducted for Trial 2 to compare SGR and FCR values for fresh and dried duckweed supplements. This test was chosen because two-sample t-tests are useful for showing differences in results of two groups. It was non-directional because the null hypothesis was that the treatments would produce similar results, not that one treatment would produce higher values of SGR and FCR.

RESULTS

Trial 1

Total and average fish body masses in each tank are shown in Appendix Tables 1-2, and feed amounts are shown in Appendix Table 3. Initial and final masses for each tank, as well as SFR and FCR, are shown in Table 1.

Table 1. Mass gain values, Trial 1

Tank	Initial Mass (g)*	Final Mass (g)*	Mass Gained (g)	SGR (%/day)§	FCR§§
Control A	2.24	10.92	8.68	4.53	1.1
Control B	1.65	7.32	5.67	4.26	1.21
Control C	1.77	8.06	6.29	4.33	1.19
30 A	1.68	5.69	4.01	3.49	1.46
30 B	2.58	5.57	2.99	2.2	2.12
30 C	1.27	4.25	2.98	3.45	1.42
50 A	1.55	3.88	2.33	2.62	1.9
50 B	1.5	3.54	2.04	2.45	2.28
50 C	1.14	3.27	2.13	3.01	1.82

* Average

§ Specific growth rate

§§ Feed conversion ratio

The mean values of SGR for the control, 30%, and 50% treatments were 4.37(0.14)%, 3.05(0.73)%, and 2.69(0.29)%, respectively (Figure 1). The mean values of FCR for the control, 30%, and 50% treatments were 1.17(0.06), 1.67(0.39), and 2.0(0.25), respectively (Figure 2).

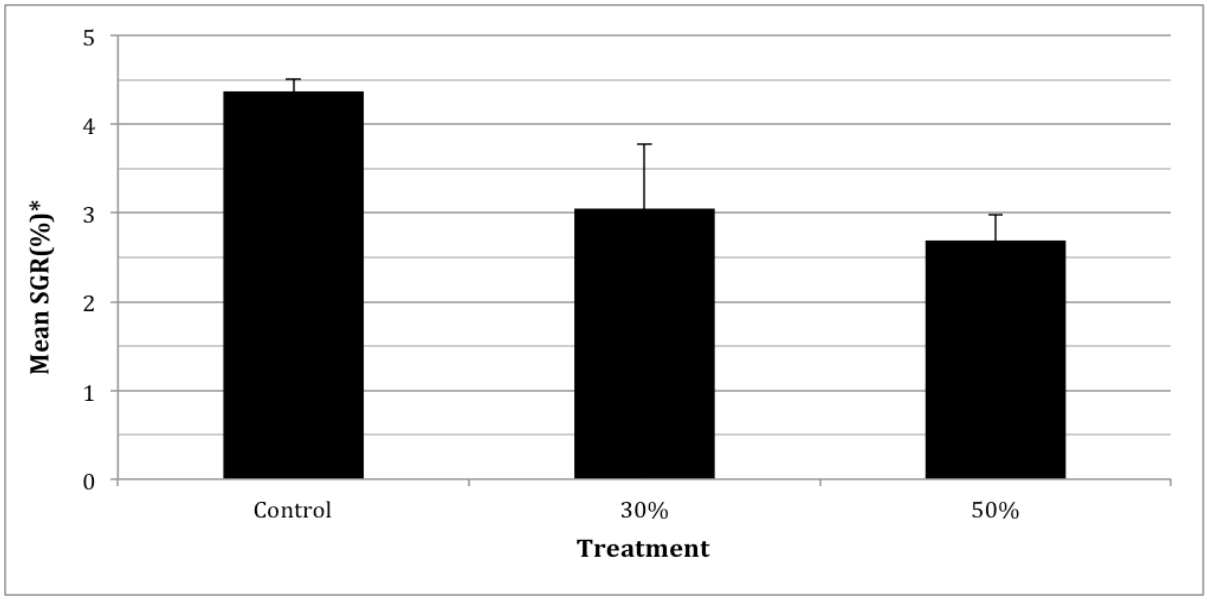

Figure 1. Mean SGR* values for each treatment, Trial 1

* Specific growth rate

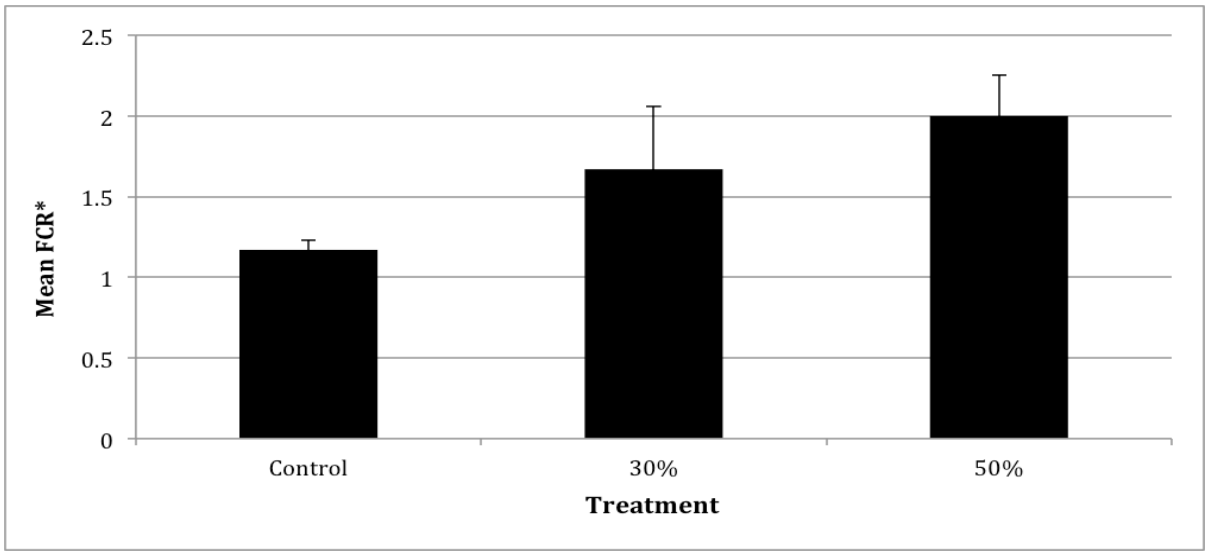

Figure 2. Mean FCR* values for each treatment, Trial 1

* Feed conversion ratio

An ANOVA test was conducted to analyze both the SGR and FCR data. In both, the treatment was used as the independent variable, while the SGR and FCR values were the dependent variables in their respective analyses.

The results of the ANOVA test for SGR indicated a significant difference among the treatments, representing a significant effect by the treatments on the SGR ($p = 0.0098$). The ANOVA test for FCR showed similar results, indicating a significant difference between FCR values based on treatment ($p = 0.025$) (Table 2).

Table 2. One-way ANOVA results, Trial 1

One-Way ANOVA Results			
Measurement	**F-value**	**$F_{critical}$**	**P-value**
SGR[§]	11.0308	5.14	0.0098[*]
FCR[§§]	7.251	5.14	0.025[*]

[§] Specific growth rate

[§§] Feed conversion ratio

* Significant

A Tukey HSD test was used for the post hoc analysis. The results of this test for SGR indicated a significant ($p < 0.05$) difference between the control and 30% treatments, as well as the control and 50% treatments. However, no significant difference existed between the 30% and 50% treatments (Table 3). These results suggest an overall decrease in SGR with increasing supplement levels, though not necessarily a linear decrease between the control and 50% treatments. By comparing the means of the control and 30% treatments, a 30.2% decrease in SGR was calculated between the two, suggesting a linear decrease is possible within this range.

The Tukey HSD results for FCR were slightly different. A significant ($p < 0.05$) difference was detected between the control and 50% treatments, but no significant difference was found between either the control and 30% treatments, or the 30% and 50% treatments. This arrangement of data, in accordance with the fact that the recorded difference between the control and 50% treatments was only slightly higher than the critical value, suggests that no considerably large gap exists between treatments for FCR, but that a small and gradual increase in FCR exists with increasing levels of duckweed supplement (Table 3).

Table 3. Tukey HSD Test results, Trial 1

Tukey HSD Test Results					
	Measurement	**Mean Comparison (g)**	**Mean Difference (g)[†]**	**Critical Value**	**P-value**
SGR[§]					
	Control - 30%	4.37 - 3.05	1.32	1.16	$p < 0.05^*$
	30% - 50%	3.05 - 2.69	0.36	1.16	$p > 0.05$
	Control - 50%	4.37 - 2.69	1.68	1.16	$p < 0.05^*$
FCR[§§]					
	Control - 30%	1.17 - 1.67	0.5	0.68	$p > 0.05$
	30% - 50%	1.67 - 2.00	0.33	0.68	$p > 0.05$
	Control - 50%	1.17 - 2.00	0.83	0.68	$p < 0.05^*$

§ Specific growth rate

§§ Feed conversion ratio

† Absolute value

* Significant

Trial 2

Total and average body masses of tilapia in each tank for Trial 2 are shown in Appendix Tables 4-5, and feed amounts are shown in Appendix Table 6. Initial and final masses for each tank in Trial 2, as well as SFR and FCR, are shown in Table 4.

Table 4. Mass gain values, Trial 2

Tank	Initial Mass (g)*	Final Mass (g)*	Mass Gained (g)	SGR (%/day)[§]	FCR[§§]
Fresh A	1.38	3.73	2.35	3.55	1.24
Fresh B	1.75	4.21	2.46	3.14	1.43
Fresh C	1.53	3.59	2.06	3.05	1.45
Dry A	1.87	4.35	2.48	3.02	1.47
Dry B	1.34	3.55	2.21	3.48	1.32
Dry C	1.58	3.64	2.06	2.98	1.47

* Average

§ Specific growth rate

§§ Feed conversion ratio

The mean SGR values for the fresh and dry treatments were 3.24(0.27)% and 3.16(0.28)%, respectively (Figure 3). The mean values of FCR for the fresh and dry treatments were 1.37(0.11) and 1.42(0.09), respectively (Figure 4).

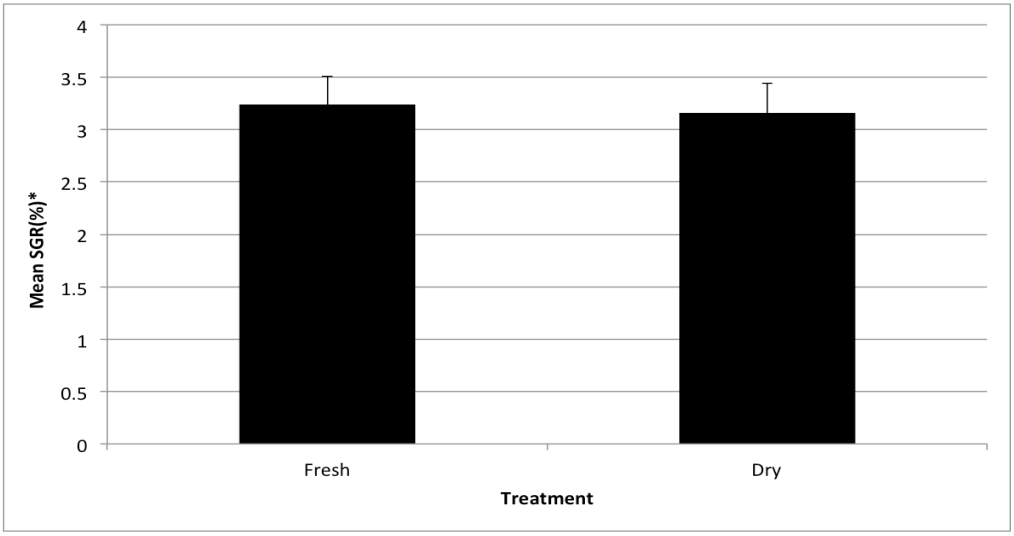

Figure 3. Mean SGR* values for each treatment, Trial 2

* Specific growth rate

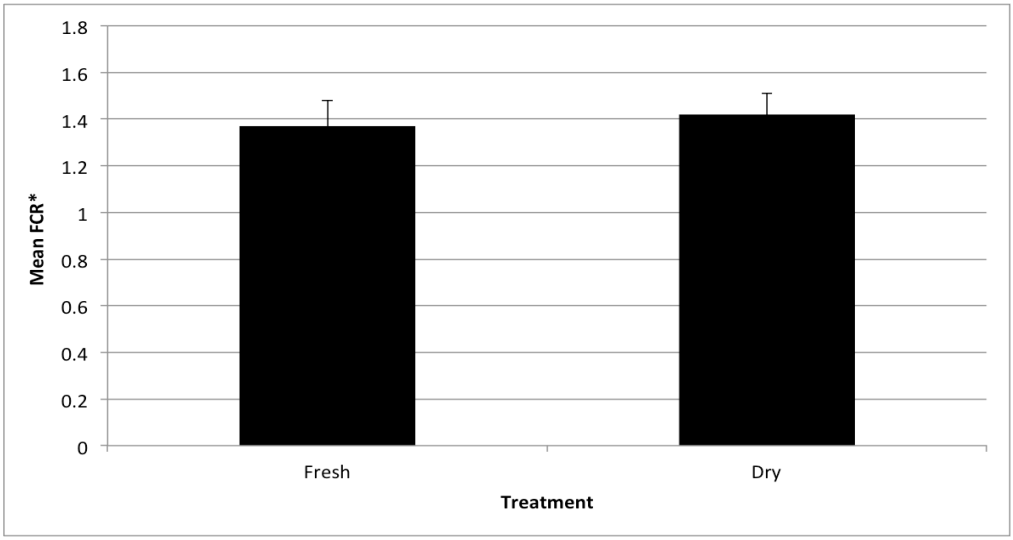

Figure 4. Mean FCR* values for each treatment, Trial 2

* Feed conversion ratio

A two-sample, two-tailed t-test was used to analyze the data from Trial 2. Results from the t-test showed no significant difference between treatments for either SGR ($p = 0.723$) or FCR ($p = 0.575$) values, indicating that fresh and dry duckweed had the same effect on growth and feed conversion of the tilapia (Table 5). No additional tests were necessary to analyze the data for Trial 2.

Table 5. Two-tailed t-test results, Trial 2

Two-Tailed T-test Results			
Measurement	**t-value**	**$t_{critical}$**	**P-value**
SGR[§]	0.38	2.776	0.723
FCR[§§]	0.61	2.776	0.575

§ Specific growth rate

§§ Feed conversion ratio

DISCUSSION

Trial 1

Results from Trial 1 indicated a reduction in SGR and an increase in FCR with higher levels of supplementation. These trends contradict the hypothesis that diets with moderate levels of duckweed supplement produce growth rates similar to those of all-pellet diets. This may mean that in commercial systems, duckweed should not be considered a reliable supplement if growth rates are to be maintained at a normal level.

There was a 30.2% decrease between the means of the control and 30% treatments for SGR, which suggests that a linear reduction is possible. It is impossible from this experiment to know with certainty what the actual progression of SGR is with respect to supplement levels between 0 and 30%. A follow-up experiment investigating levels between 0 and 30% could be useful for getting a better idea of the actual trend. If, in such an experiment, the results did suggest a linear 1:1 decrease in SGR with increasing supplement levels, this information could prove very useful to both commercial fish farmers and hobbyists. For people or companies looking to be more sustainable and potentially save money, a balance between slightly higher supplement levels and slightly lower growth rates could be tailored individually to meet the needs of the particular person or company if a 1:1 relationship were established. As an example, a fish farmer may be comfortable lowering the SGR of his tilapia by 5%, if as a result he could guarantee an equivalent change in the sustainability and cost of his system. A 1:1 relationship would allow people to adjust the supplements according to their needs.

The FCR did not show the same gap between the control and 30% treatments as was seen in the SGR analysis. While a difference was detected between the control and 50% treatments, the difference between the control and 30% treatments was not significant. This information could also be useful when considering small supplements such as those suggested in the case of a 1:1 relationship between supplements and SGR. If a person chose to compromise SGR slightly by using limited supplemental duckweed, he could reasonably assume that he was not also compromising the conversion efficiency of his fish. This would mean food would not be wasted in high FCR values.

Tilapia typically have some of the best FCR values of all farmed animals, usually between 1.4 and 1.8 (DeLong et al., 2009; FAO, 2006). Although the FCR increased over the

treatments in this experiment, the highest FCR mean (that of the 50% treatment) was 2.0 and the 30% mean was 1.67. These results suggest that even though FCR worsened with increased supplements, the highest FCR observed over the treatments was only slightly higher than the typical values for tilapia, and the 30% treatment produced values well within the standard range. It is also important to note that a FCR of 2.0, although higher than average for tilapia, is still one of the lowest in agriculture and aquaculture. As a comparison, the FCR for beef cattle generally falls between 7 and 25 (Coffey, 2015). The data suggest that even with quite large levels of supplemental duckweed, the FCR remains very good.

Trial 2

Based on results from Trial 2, the null hypothesis that fresh and dry duckweed would produce similar SGR and FCR values could not be rejected. Therefore for this study it is appropriate to accept that the type of duckweed given to the fish, either fresh or dry, did not affect growth or feed conversion.

There was only a 2.5% difference between the SGR values of the treatments, and a 3.6% difference between the FCR values. These results suggest that a fish farmer who is incorporating duckweed into an aquaponics system could use either fresh or dry plants and assume the same results for both. This could be very important, especially for people who do not have year-round access to warm weather or a greenhouse. The possibility of using dry duckweed means that large quantities can be grown, either during favorable seasons or indoors, and then dried and stored for later use, during the winter season for example. If fresh duckweed were necessary for good results, production could be impossible during colder months. Therefore, the results of Trial 2 are very favorable to those considering duckweed supplementation in aquaponics systems.

Limitations

Several limitations were encountered during the trials. Although it was assumed that the amount of food consumed was approximately equal to the amount of food added to the tanks, there was no definitive way to quantify the actual amount of food consumed by the fish. A change in that value would lead to changes in the FCR of the treatments and produce a more accurate calculation. Additionally, the precision of the scale used to mass the pellets and duckweed only allowed accurate measurements to the nearest 0.1 gram. This sometimes led to

rounding, which altered supplement levels slightly, by less than 0.1 gram. However, due to the size of these units and the fact that the FCR values were very similar to those expected, it is likely that these limitations were not a significant issue.

Another potential limitation of the experiment was fish mortality in three of the tanks during Trial 1. One tank in each treatment had a loss of one fish during the trial. The use of average mass to calculate SGR and FCR was intended to allow analysis even with fish mortality, but a truly accurate measurement would require all fish to survive through the trial, assuming the deceased fish did not have the exact same mass as the average mass. It is unclear why three fish died, as all other fish in the tanks appeared healthy and normal, so this limitation would be difficult to correct in future experiments. There was no fish mortality in the second trial, so this limitation did not apply to the results from Trial 2.

Water quality can be a serious issue in many aquaculture systems and could be a potential limitation in this experiment, as there were no methods to address the measurement and control of water quality. Although no measurements were made, it was noted that the water in all three control tanks in Trial 1 appeared cloudier than the water in both other treatments. Two possible explanations for the cloudy water are that the fish in the control tanks were generally larger than in the other treatments, leading to larger food inputs and increased waste, or because of the known water-cleansing properties of duckweed, which was present in both other treatments (Edwards et al., 1992). The latter explanation is unlikely, as the duckweed in the 30% and 50% treatments was rarely in the water for more than a few minutes without being consumed. While it is unclear exactly why the control tanks were slightly cloudier than the others, it is possible that poorer water quality in these tanks affected growth. However, as these tanks still produced the highest SGR values and lowest FCR values, it is unlikely that the clouded water affected their growth considerably. Another water quality issue to consider is dissolved oxygen, which DeLong et al. (2009) stated could affect growth rates if not kept at proper levels. While it must be noted that dissolved oxygen could play a role in the SGR and FCR values recorded in this experiment, DeLong et al. (2009) estimated that in well-aerated systems, the maximum biomass of fish should not exceed 0.25 lb per gallon of water in order to avoid compromising growth rates. Because this estimated limit was not exceeded in this experiment and adequate oxygen was provided, it is unlikely that dissolved oxygen played a major role in SGR or FCR values.

One of the most important limitations of this study was the lack of data on the sexes of the tilapia in each tank. It is known that female tilapia do not grow as quickly as males because they must put energy into egg production. It is estimated that when males and females are grown together in the same system, the females will be half as large as the males (DeLong et al., 2009). The sexes of tilapia in both Trial 1 and Trial 2 were not able to be determined, and therefore it is likely the ratio of males to females across tanks varied. In this case, a tank with mostly males may show high growth rates, while a tank with mostly females may show low growth rates, even if they are included in the same supplemental treatment. While this is a serious limitation to consider, Palada-de Vera and Eknath (1993) compared male and female growth rates in seven strains of Nile tilapia (*O. niloticus*) over time and found that growth rates between males and females did not become considerably divergent until between 108 and 129 days after birth. Based on the mean weights of Nile tilapia in the study by Palada-de Vera and Eknath (1993) at 150 days and the size at which the fish in the current experiment were obtained, it is likely that the fish used in this investigation had not yet reached 108 days or experienced considerable size divergence. Even with this information, male-to-female ratios in the tanks must still be considered as a possible limitation.

Recommendations for Allegheny College and Future Studies

It is clear that in large quantities, duckweed may negatively affect SGR and FCR values for Blue tilapia, and therefore may not be desirable to commercial fish farms that require optimum growth rates in order to supply large tilapia to the market. Although these companies may choose to use duckweed supplements in small quantities to lessen environmental impact or lower costs, duckweed supplementation at levels at or above 30% would not be recommended to these organizations based on the data collected, as their profits depend largely on very high SGRs and very low FCRs. There may be slightly more leeway for recommendations to fish farmers who sell fry or fingerlings because these fish do not necessarily need to grow as large or as quickly, and require many smaller feedings throughout the day. Replacing one or two of these feedings with a handful of duckweed instead of fish pellets could potentially reduce costs and impacts without sacrificing necessary body mass.

Though a recommendation of 30% or more duckweed supplement to companies raising tilapia for human consumption may not be appropriate, the Environmental Science department at

Allegheny College operates a very different system and may benefit from such a recommendation. Although the tilapia in the aquaponics system at the College are harvested once each year for the Jennifer DeHart local foods dinner, their main purpose is to be an educational and hands-on project within the department. The system is helpful for teaching students about environmental impacts caused by the food fish industry, including aquaculture, as well as ways to improve the sustainability of food production, such as eating locally grown foods and foods not treated with harsh pesticides or fertilizers. In addition to being an educational tool, the system provides enjoyment to those working in the lab and jobs for work study students. The lettuce grown from the tilapia wastewater is also harvested for consumption at school. Despite playing a small role as a supplier of tilapia to the local foods dinner, the main purpose of the system relies very little on producing large fish in a short amount of time. Because SGR and FCR matter very little in the context of this aquaponics system in particular and duckweed supplements still maintained decent values in the study, it is recommended that the Environmental Science department try to incorporate moderate levels of supplemental duckweed into the system.

These supplements would be time-consuming to actually measure precisely, so it is unlikely at this point that an exact measured supplement will be included in the fishes' diet. A recommendation with this in mind would be to cut back slightly on the amount of pellets given at each feeding and replace it with a handful of duckweed. For example, instead of providing the fish with a full 400 ml beaker of pellets at each feeding, the caretaker could fill the beaker to 80% capacity, and add a handful of duckweed.

Based on results from Trial 2, the duckweed incorporated into the system could be dry. This decreases the work time needed for each feeding, as it would not be necessary to harvest duckweed each time. Instead, large quantities of grown duckweed could be harvested over long time intervals and left to air dry. The dry duckweed could then be kept in a container for feeding, just like any other dry fish food. For each feeding, the caretaker would only need to grab a handful of duckweed from the food container. If the duckweed plants were being grown in a relatively small area, performing large harvests could also possibly allow the existing growing duckweed to spread more quickly, as space could be a limiting factor for such a fast-growing plant. Therefore, it is recommended that duckweed be dried for incorporation into the aquaponics system at Allegheny College.

The biggest hurdle to overcome before this plan could be implemented is the issue of growing duckweed at the school. For the current experiment, small amounts of duckweed were required, so it was simple to grow duckweed in water-filled bins. On a larger scale, however, it would be necessary to establish a consistent means of growing large amounts of duckweed. Fortunately, duckweed grows rapidly and easily. Space would be the largest issue.

These are ideal issues to address in future senior projects. One possibility would be trying to incorporate duckweed growth into the system itself. Some do-it-yourself aquaponics hobbyists have incorporated homemade growing rafts that float on the surface of the water and hold duckweed within a frame. A mesh barrier 1-2 inches below the water prevents the fish from simply eating all the duckweed while it grows. This would save space and provide additional water-purification for the system, while allowing relatively large amounts of duckweed to grow. Another possibility would be using a mesh wall to separate part of the large aquaponics tank, with fish on one side and duckweed on the other. One downside to this technique would be the loss of space for the fish. These methods of duckweed growth could both be tested for practicality at Allegheny through senior projects, and if successful, could pave the way for duckweed incorporation into the system.

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APPENDIX

Appendix Table 1. Total fish body mass per tank, Trial 1

	Total Mass (g)					
Tank	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Final**
Control A	22.4	37.3	48.6	64	78.5	98.3
Control B	16.5	28.3	38.1	51.6	61.6	73.2
Control C	17.7	31	42.2	55.5	66.6	80.6
30 A	16.8	26.2	33	42.8	48.7	56.9
30 B	25.8	26.8	30.3	39	43.8	50.1
30 C	12.7	19.2	22.2	30.5	36.5	42.5
50 A	15.5	18.7	22.4	27.5	31.2	34.9
50 B	15	25.5	24.5	31.6	36.3	35.4
50 C	11.4	20.7	22.6	25.9	30.2	32.7

Appendix Table 2. Average fish body mass per tank, Trial 1

	Average Mass (g)					
Tank	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Final**
Control A	2.24	3.73	5.4	7.11	8.72	10.92
Control B	1.65	2.83	3.81	5.16	6.16	7.32
Control C	1.77	3.1	4.22	5.55	6.66	8.06
30 A	1.68	2.62	3.3	4.28	4.87	5.69
30 B	2.58	2.98	3.36	4.33	4.87	5.57
30 C	1.27	1.92	2.22	3.05	3.65	4.25
50 A	1.55	2.07	2.49	3.05	3.47	3.88
50 B	1.5	2.55	2.45	3.16	3.63	3.54
50 C	1.14	2.07	2.26	2.59	3.02	3.27

Appendix Table 3. Fish diets[§], Trial 1

	Mass of Feed (g)									
Tank	Week 1		Week 2		Week 3		Week 4		Week 5	
	P*	D**	P	D	P	D	P	D	P	D
Control A	1.1	0	1.9	0	2.4	0	3.2	0	3.9	0
Control B	0.8	0	1.4	0	1.9	0	2.6	0	3.1	0
Control C	0.9	0	1.6	0	2.1	0	2.8	0	3.3	0
30 A	0.6	0.3	0.9	0.4	1.2	0.5	1.5	0.6	1.7	0.7
30 B	0.9	0.4	0.9	0.4	1.1	0.5	1.4	0.6	1.5	0.7
30 C	0.4	0.2	0.7	0.3	0.8	0.3	1.1	0.5	1.3	0.5
50 A	0.4	0.4	0.5	0.5	0.6	0.6	0.7	0.7	0.8	0.8
50 B	0.4	0.4	0.6	0.6	0.6	0.6	0.8	0.8	0.9	0.9
50 C	0.3	0.3	0.5	0.5	0.6	0.6	0.6	0.6	0.8	0.8

§ per day

* Pellets

** Duckweed

Appendix Table 4. Total fish body mass per tank, Trial 2

	Total Mass (g)				
Tank	Initial	Week 1	Week 2	Week 3	Final
Control (Fresh) A	13.8	17.4	22.7	29.5	37.3
Control (Fresh) B	17.5	22	26.4	34.7	42.1
Control (Fresh) C	15.3	18.8	22.7	28.3	35.9
Dry A	18.7	22.5	28.1	34.8	43.5
Dry B	13.4	17.7	23.6	28.8	35.5
Dry C	15.8	19.3	22.9	28.6	36.4

Appendix Table 5. Average fish body mass per tank, Trial 2

	Average Mass (g)				
Tank	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Final**
Control (Fresh) A	1.38	1.74	2.27	2.95	3.73
Control (Fresh) B	1.75	2.2	2.64	3.47	4.21
Control (Fresh) C	1.53	1.88	2.27	2.83	3.59
Dry A	1.87	2.25	2.81	3.48	4.35
Dry B	1.34	1.77	2.36	2.88	3.55
Dry C	1.58	1.93	2.29	2.86	3.64

Appendix Table 6. Fish diets[§], Trial 2

	Mass of Feed (g)							
Tank	**Week 1**		**Week 2**		**Week 3**		**Week 4**	
	P*	**D****	**P**	**D**	**P**	**D**	**P**	**D**
Control (Fresh) A	0.5	0.2	0.6	0.3	0.8	0.3	1.0	0.4
Control (Fresh) B	0.6	0.3	0.8	0.3	0.9	0.4	1.2	0.5
Control (Fresh) C	0.5	0.2	0.7	0.3	0.8	0.3	1.0	0.4
Dry A	0.7	0.3	0.8	0.3	1.0	0.4	1.2	0.5
Dry B	0.5	0.2	0.6	0.3	0.8	0.4	1.0	0.4
Dry C	0.6	0.2	0.7	0.3	0.8	0.3	1.0	0.4

§ per day

* Pellets

** Duckweed