# Kampachi Farms NOAA SK Final Report

**Project Title:** Herbivorous marine finfish culture- the compelling case for kyphosids

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## Final Report Summary

This project investigated a new family for the nascent U.S. marine finfish aquaculture industry, Kyphosidae, that could potentially be grown independently of fishmeal and fish oil resources. Research assessed the feasibility of this new species to commercial aquaculture - can it be raised to market size on commercially available feeds; and, does the end product have a beneficial fatty acid profile, and is it palatable to consumers?

# Table of Contents

# 1. Background

Why Kyphosids?

The conventional wisdom has been that marine fish are either carnivorous and tasty (groupers, snappers, Kona Kampachi ® and other yellowtails), or else are herbivorous and less desirable (milkfish, mullet). Amongst the profusion of coral reef fish species, however, there are several groups of herbivores that are well regarded as food fish. Kampachi Farms' principals and associates have collectively spent decades working throughout the developing world, in the Pacific Islands, SouthEast Asia, Latin America and Africa, and have had ample opportunity to sample a wide variety of coral reef fishes from subsistence and artisanal fisheries. From among these, we have identified a hierarchy of palatability for a range of potentially cultured species.

There are four highly desirable groups of herbivorous marine fishes: parrotfish (*Scarus* spp), unicornfish (*Naso* spp), rabbitfish (*Siganus* spp) and rudderfish ( *Kyphosus* spp). Parrotfish and unicornfish are both difficult to rear as larvae, with very small eggs. Kona Blue Water Farms (the precursor to Kampachi Farms) and a number of other researchers in Hawai'i have strived in the past to rear a close relative of *Naso*: the yellow tang (*Zebrasoma flavescens*). The difficulty in raising larvae of this species is generally attributed to the small egg size, and consequently small larvae at first hatch. Recent success by the Oceanic Institute in rearing a limited number of these fish may lead eventually to success in similar species, but their culture remains challenging in the main. Parrotfish are protogynous hermaphrodites, and the flesh quality only reaches its optimum in larger males; females are smaller, and less palatable. Unicornfish also possess razor sharp caudal spines that make them difficult to handle and to work with in a net. Rabbitfish have larger eggs, but have the challenge of venomous spines. They are also not native to Hawai'i and most of the Eastern Pacific; culture of non-native fish would not be permitted in offshore cages under mariculture regulations in the Western Pacific (WESPAC Region) and the Gulf of Mexico (GMRFMC Region). The kyphosids, however, present none of these impediments.

Three species of kyphosids (collectively 'nenue' in Hawaiian) are known from Hawaiian reefs: *K. cinerascens* (Highfin Chub), *K. biggibus* (Gray Chub) and *K. vaigiensis* (Brassy Chub). The Highfin is known among fishermen to be less palatable – a tougher, drier flesh. The Brassy Chub is both highly regarded and generally more common, and is readily collected from a 'koa' or fishing hole at Keahole Point (the location of Kampachi Farms' facility). Accordingly, *K. vaigiensis* will be the focus for this project.

Kyphosids have a broad range of traits that are amenable to aquaculture:

Ubiquity: Kyphosids are found throughout most of the temperate and tropical waters of the world (Randall et al., 1990), and have the potential to be widely cultured. The Brassy Chub, *K. vaigiensis*, has a wide distribution throughout the IndoPacific, including Hawai'i.

Herbivory and Digestive Capabilities: Kyphosids are classified as "true" herbivores (Choat, et al., 2004) and are known, through gut content analyses to consume a wide variety of algal types including red, brown, and green algae (Silvano and Guth, 2006;

Moran and Clements, 2002). An exceptionally long hindgut in the digestive tract (Rimmer and Wiebe, 1987) makes kyphosids well suited for plant digestion. Some of the algal species that kyphosids consume are troublesome invasive species to Hawai'i and other parts of the world. Harvesting and utilizing these algae as future fish food ingredients could help control these harmful species.

At a minimum, kyphosids should be able to digest agricultural grains, such as soybean meal, wheat and corn products, in less expensive, less processed forms than other 'carbohydrate-intolerant' marine fishes. Kyphosids produce digestive enzymes capable of uptaking algal polysaccharides (Montgomery and Gerking, 1980; Horn, 1989; Moran and Clements, 2002). Of all the polysaccharide substrates in algae (starch, laminarin, carrageenan, alginate and agarose), kyphosid digestive enzymes most readily utilize starches (Skea et al., 2005). Although the cell wall composition will differ in agricultural grains and algae, the preferred uptake of algal starches by endogenously produced enzymes in kyphosid intestinal tissue is an encouraging prospect for their acceptance of agricultural starches. In addition to this digestive pathway, kyphosids also rely on fermentation in their hindgut in order to utilize substrates from alga which they can not uptake directly (Clements and Choat, 1995; Skea, et al., 2005; White, et al., 2010). Hindgut fermentation gives kyphosids access to components of brown algae, such as sugar alcohols, that otherwise could not be uptaken due to lack of intestinal transporters (White et al., 2010). This fermentation-aided component of kyphosid digestion may allow them access to greater amounts of nutritive substrates in agricultural grains, similar to ruminant livestock. Ruminant-like microbial fermentation converts dietary fiber (mainly of plant cell wall carbohydrates) into short chain fatty acids (SCFA). Kyphosids have among the highest levels of SCFA recorded in the gut of fishes (Clements and Choat, 1997). This aptitude for digesting complex carbohydrates further supports the belief that kyphosids should be able to digest a wide variety of plant materials, both terrestrial and marine, allowing for cost effective feed formulations with less processing, and without reliance on fishmeal and fish oil.

<u>Docility:</u> Kyphosids are schooling species that do not appear to display aggressive intraspecific behavior, but thrive well in captivity. Both Waikiki Aquarium (Jerry Crow, pers. comm.) and Maui Ocean Center (John Gorman, pers. comm.) have maintained kyphosids in large and small tanks for years, with no problems with aggression or diseases. An individual *K. vaigiensis* serendipitously recruited naturally into Kampachi Farms' Velella pen – an untethered open ocean cage trial – where it thrived alongside 2,000 amberjack "penmates".

<u>Parasite</u> <u>Resistance:</u> Kyphosids are covered with small, ctenoid (toothed) scales that may render them more resistant to ectoparasites than other marine fish. The single recruit into the Velella pen showed no *Neobenedenia* skin flukes or other ectoparasites at all, while the kampachi cohabiting the pen were infected at levels ranging from 0.7 – 5.0 flukes per fish.

To further examine the resistance of kyphosids to skin fluke parasites, Kampachi Farms conducted a trial wherein a tank containing juvenile kampachi and *K. vaigiensis* was deliberately exposed to fouled mesh harboring *Neobenedenia* eggs for a period of one month. At the end of the trial, the kampachi in the tank hosted an average of 65 flukes each, while the kyphosids were completely unaffected. Although more testing will be required to determine the full extent of the ability of kyphosids to resist parasite infestation, it appears that many of the concerns regarding disease and parasite management (and the attendant significant expenses and inefficiencies) for other species may not apply in the culture of kyphosids.

Growth: Kyphosid growth rates are commercially attractive: the fishing records for these species (Hawaiian nenue = 3.3 kg, or 7.4 lbs, Bermuda Chub = 6 kg, or 13 lbs: Fishbase) imply good growth rates over the initial year of culture. The kyphosid that recruited into the Velella pen attained a size of 965 g (2.1 lbs) after six months of feeding alongside the kampachi (*Seriola rivoliana*). This fish yielded 48% to skin off fillet (rib bones out, pin bones in); the same yield as kampachi. Preliminary tank trials at Kampachi Farms' facility indicate an average growth rate from 100 g to 1 kg (1/4 lb to over 2.5 pounds) in 12 months on a mixed diet that included pellets of Skretting "Kona Pacific" diet (48% protein, 24% lipid), and a commercial gel diet that was 18.5% crude protein and 1.2% fat.

Kyphosid growth is also efficient: tank trials with the mixed diet at the Kampachi Farms facility yielded a Feed Conversion Ratio (feed pellet weight: wet fish weight) of 1.77 over a four month period (n = 12 fish: 10/2011 – 1/2012). This FCR is encouraging for an initial trial, and could be further improved with refinements to diet and feeding strategies: e.g. as an herbivore, kyphosids probably function more efficiently on regular, frequent rations, perhaps five or more times per day, rather than the single daily feeding that is SOP for most adult carnivorous fish.

Amenability to hatchery culture: Eggs from wild-collected kyphosids in Hawai'i are relatively large: around 900 um diameter. This is similar in size to eggs of *S. rivoliana* (Kona Kampachi®), which Kona Blue Water Farms has been able to produce in mass quantities in commercial hatchery contexts. Egg size is the best determinant for larval yolk reserves and size at first feeding, which are the best indicators for the type of live feeds that the first feeding larvae will accept. The large size of kyphosid eggs strongly supports the presumption that kyphosid larvae will readily take a rotifer at first feeding (rather than the additional challenge of culturing copepods or other, smaller, first feeds), enabling standard marine fish culture techniques to be applied. During research trials in 2004 with rustic "greenwater mesocosm" larval culture systems, Kona Blue Water Farms successfully reared larval *Kyphosus* sp. through to metamorphosis stage (Figure 1) from eggs collected by filtering incoming seawater. There was good survival of the larvae, and juveniles were weaned and raised up to 4 cm length on the first attempt. This demonstrates clearly the aptitude of these fish to larval culture.

Figure 1: A weaned juvenile *Kyphosus* sp. reared by Kona Blue researchers in 2004.

Palate appeal: These circum-tropical, marine herbivorous fishes are targeted as a food fish in coral reef fisheries throughout Hawai'i (Sakihara et al., 2014), other Pacific Islands (Atu, 2005) and Southeast Asia. Kyphosids are an esteemed commercial and recreational fish, with a price index of "HIGH" (Fishbase), but are not targeted by commercial fisherman, so culture of these fish would not threaten fishing community incomes. Kyphosids are consumed in a variety of preparations, ranging from whole steamed or roasted fish, and standard fried fillets, to a more exotic raw fish salad (poke) and as smoked fish.

The natural recruit to the Velella pen had a fat content of 29% (versus around 33% for the kampachi from the same pen). This fish was sampled by renowned national Chef Sam Choy, who "loved the fish …. loved the fattiness of it", and served small sashimi samples to guests in his Kona restaurant (in litt., Sam Choy, 1/27/2012).

In some parts of the world, such as Australia and the Southern USA, these fish and their close relatives are less well regarded, because their herbivorous habits give them a strong algae/ fishy taste to the flesh. In Australia and New Zealand, however, this flavor may be a result of the very high levels of phlorotannins in the fucoid algae that are a prime constituent of kyphosid diets (Russell, 1983; Horn, 1989). Chef Choy was pleased to find "no ogo (algae) taste to the fish" that he sampled from the Velella project. With careful tuning of commercial kyphosid diets, the flesh texture and taste could be made highly desirable.

The experience of Kona Blue cofounders (Sims and Sarver) with cultured kampachi compared with wild kahala (both *S. rivoliana*) demonstrates the extent to which taste, quality, reputation and price point of a cultured fish is a function of its diet. Wild kahala has lipid levels of around 3-4%, yet when cultured in tanks or pens on a formulated diet, this could be increased to over 30%. This increase in lipids (including heart healthy omega -3 fatty acids) rendered what was a reasonably palatable wild product (albeit one plagued with parasites in the flesh, and a history of ciguatera) into Kona Kampachi®: a brandable, trademarked sushi grade fish that was accorded accolades at the highest level, culminating in its being awarded "Fish of the Year" honors by Food Arts magazine in 2008. We believe that the evidence accumulated to date strongly suggests that farm raised kyphosids can launch onto a similar trajectory, and become a high value, highly marketable product.

## 2. Objectives

This work tested whether or not commercially available diets (tilapia or marine grower pellets) produce economically attractive growth rates and FCR, and desirable taste and fatty acid profiles for kyphosids. Additionally, an experimental algae-based macroalgae pellet was formulated and tested for application in marine herbivore culture. Through a community partnership with Paepae o He'eia, the project also established a trial to test the growth, taste, and fatty acid profile of kyphosids grown on invasive algae. The final product of research is an assessment of the viability of kyphosid culture on readily available feeds. This study also provides an indication of how feed formulations could be refined to improve successful culture of this species.

The specific Goals and associated questions of the project are:

1. Grow kyphosids on feeds readily available on the market.
    a. Will a commercially available diet produce economically attractive growth rates and FCR?
    b. Does the tilapia diet perform similarly in growth and FCR to the trout diet?
    c. Is the fatty acid profile of kyphosids grown on commercial diets similar to carnivorous finfish?
    d. Does the tilapia diet produce a similar fatty acid profile to the trout diet?
    e. What is the difference in feed cost? Can this be extrapolated to commercial scale savings?
    f. Which feed treatments produce the most desirable taste and texture for human consumption?
2. Evaluate the ability of kyphosids to consume invasive algae.
    a. Have they grown at a reasonable rate to confirm they can be sustained on an invasive algae diet?
    b. Is the fatty acid profile of the fish attractive for consumption as a healthful source of omegas?
    c. Do the fish have a desirable taste and texture for human consumption?
    d. What range of salinity were the fish able to tolerate during the grow out period?

This final report is divided into sections of commercial diets, algae-based diets, and community work with Paepae o He'eia under each major report heading to address these goals.

# 3. Methods and Materials

## 3.1 Growth on Commercially-Available Pelleted Diets

### 3.1.1 Marine Grower Trout Diet vs. Tilapia Diet

On November 4th, 2016, at the Kampachi Farms facility, forty juvenile kyphosids (start weight 386 ± 78 g) were evenly and randomly distributed into four, 4-tonne HDPE tanks, held on flow-through of surface seawater at ambient temperature and photoperiod. The fish were individually PIT-tagged, weighed, and measured for fork length at the initial weighing event. A marine grower Steelhead trout diet (45% protein, 16% lipid) and a tilapia diet (35% protein, 5% lipid) were each randomly assigned to half the tanks.

Weighing events were conducted every month, where individual weights and lengths were taken to track individual fish growth. In December 2016, all tanks were moved onto belt feeders, which dispersed pellets throughout the daylight hours. It was hypothesized that switching to belt feeders would increase consumption / growth by providing consistent food availability, akin to how kyphosids graze in the wild. The mass of feed per belt feeder started at 100g for each tank, representing roughly 200% of the average daily feed consumed during the preceding batch feeding regimen. It was then down-adjusted per tank to curb the accumulation of uneaten food. Ultimate weight for the belt feeders were approximately 150% of the average daily batch feeding weight.

The trial concluded on May 4th, 2017. All fish were weighed and three fish from each treatment were harvested for taste-testing and lab analyses (proximate and fatty acid). Blind taste-testing was conducted on a small scale - with 7 participants grading raw fillets (prepared sashimi style) on texture, appearance, fat content, algae flavor, and overall taste. Flesh samples for proximate and fatty acid analysis were taken from the top side of the fillet closest to the head of the fish, and analyzed at Eurofins Scientific in Iowa.

### 3.1.2 Capturing the Growth Curve

In order to capture as much of the juvenile growth curve as possible, we opportunistically collected growth data from the small numbers of kyphosids of less than 50g that were been obtained from offshore collections near floating objects or moorings.

Beginning December 22$^{nd}$, seventeen kyphosids (start weight 2.9±1.7g) were randomly split for comparative rearing. The trial ran for 63 consecutive weeks. Upon trial start, fish were randomly sorted into two tanks: 9 fish (average 2.7 ± 1.8g) on a tilapia diet (TD; 35% protein, 5% lipid), 8 fish (average 3.1 ± 1.7g) on a marine grower diet (MG; 45% protein, 16% lipid). Fish were fed 5 times daily for the first six months, then switched to 3 times daily for the remainder of grow-out; fish were weighed every 2 weeks throughout the duration of the trial.

For the first 22 weeks, feed weights were not recorded as feed had to be pulverized and it was not feasible to record feed waste. At the week 24 weighing event, all fish were capable of taking a whole 3.2mm pellet, and feed consumption and waste was recorded for calculations of FCR.

Viscera from three fish in each treatment were analyzed for fat content at the harvest event. Measurements included total body weight (bw), total visceral weight, and the weight of the fat surrounding the viscera (separated by hand). No gonads were present for measurement. From the fat measurements, a viscerosomatic index (VSI), % fat of viscera, and % visceral fat:bw ratio were calculated by diet.

By following growth of post-larval kyphosids over the period of approximately one year, we have collected information about the commercial grow-out cycle from hatchery-size fingerlings to a suitable harvest weight.

## 3.2 Algae Diets for Marine Herbivorous Finfish

In the interest of testing kyphosids on algae-based feeds in a trial setting to inform the best feed formulation to pursue in further growth-trial iterations, two parallel avenues of research were conducted:

a. Sourcing locally-grown macroalgae
b. Formulation of an algae-based pelleted diet

### 3.2.1 Wild Limu Diet

A 12-week growth trial of kyphosids on wild-sourced macroalgae (limu) was conducted to provide a baseline of growth on a 'natural' diet. A total of nine fish were individually tagged and stocked into a 4-tonne HDPE tank held on flow-through surface seawater at ambient temperature and photoperiod. Limu was stocked in a wall-hanging net structure every 2-3 days as they grazed it down.

The varieties of algae were mostly *Sargassum* (found in abundance in the stomachs of wild kyphosids collected for gonads for another project), and also *Chnoospora*. Algae was collected weekly and stored in a 100 L holding basin on site, fed with a flow-through of surface seawater at ambient temperature and photoperiod. The fish were weighed monthly, and individual weights (identified by PIT tag) were used to track growth performance.

### 3.2.2 Algae-Based Pellets for Kyphosids

Two iterations of an algae-based pellet (composition: 54% *Gracilaria*, 25.7% *Sargassum*, 15% wheat flour, and trace vitamins and minerals) were formulated by Dr. Rick Barrows. The first iteration of the diet was milled with fish oil included in the base mixture, and shortly after arrival at the Kampachi Farms' site was found to have gone rancid. The onset of rancidity was much faster than has been seen in any other experimental formulation. Dr. Barrows hypothesizes that this is due to the interaction of the oil with the high ash content in the algae component of the diet, which can cause rapid peroxide production.

The second iteration was milled as a dry pellet, to be regularly top-coated with the necessary fish oil at the Kampachi Farms site, to circumvent the rapid onset of rancidity. Further testing showed that even this pellet will still go rancid if the mixture is left to sit for approximately one week in the refrigerator. After mixing, the oil began oxidizing within two hours after being coated onto the pellets, and at 3 days after mixing the diet was unusable (Figure 2). The revised feed preparation protocol included daily mixing of the pellets and the oil. This understanding of the stability of high-oil formulations with macroalgae could be very useful for future improvements to formulation of macroalgae based diets.

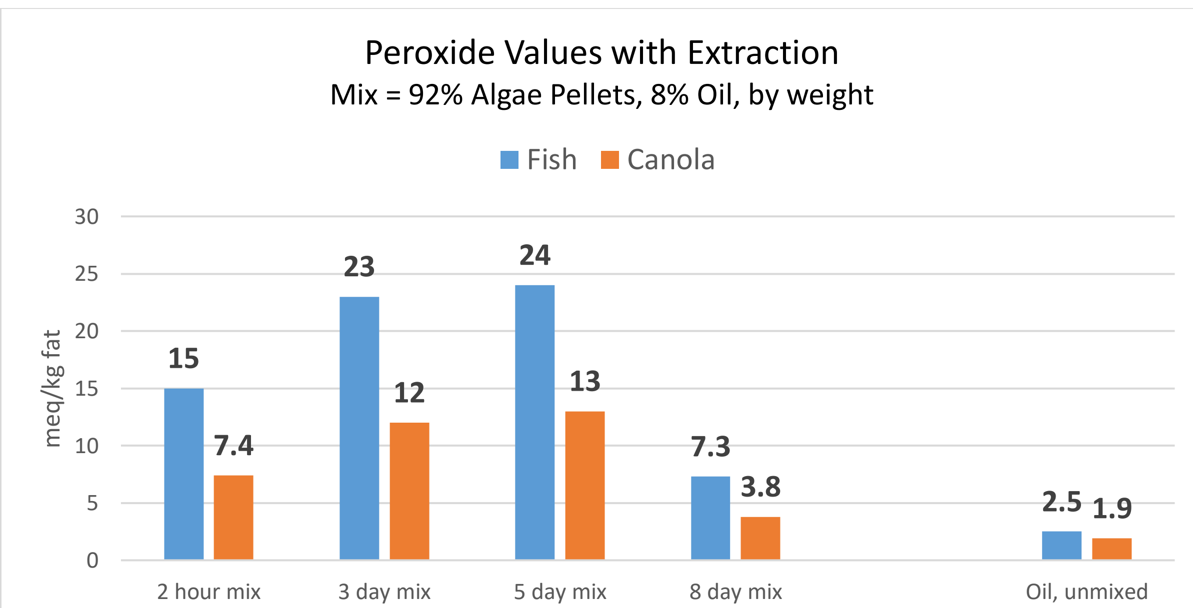


*Figure 2: Peroxide values of algae-based pellets that were top coated in oil and kept in a refrigerated storage for x number of hours/days before processing. A peroxide value of 20 meq/kg of fat indicates rancidity that renders the diet unusable.*

The fish used to stock this trial were re-randomized from the tilapia diet vs. marine grower trial conducted in 2016-2017, and were held on the control pellet (tilapia diet) prior to trial start. After fielding the rancidity hurdles with the algae-based pellet, a feed trial was initiated.

On November 8, 2017, twenty-nine kyphosids at an average weight of 610 ± 105 g were randomly distributed into six tanks to provide three replicates of each treatment. All fish were fed to satiation three times daily and weighed monthly. At week 12, the 6-tank configuration was consolidated into one tank for each treatment due to consistently low overall feed consumption and negative growth. This allowed indication of whether or not being stocked at greater density would improve feed consumption, as kyphosids are often found in large schools. The fish were fed three times daily to satiation by hand, and weighed monthly throughout the trial time period.

### 3.3 Invasive Algae Acceptability

Paepae o He'eia, stewards of He'eia fishpond on Oahu, were subcontracted to conduct an invasive algae acceptability test – feeding wild-caught kyphosids (known as nenue or enenue in Hawaiian) one of three species of invasive algae found locally (*G. salicornia, A. specifiera, and K. alvarezii*). Following grant dispersal, a Kampachi Farms staff member travelled to Oahu to assist in building and siting their first cage in the pond to house their experimental fish (Figure 4). Unfortunately, there was a long period of time where wild-caught nenue were not able to be sourced from their local contacts. To increase their motivation to catch fish for the pond, we increased the reward per fish from $15 to $25. We provided guidance on the methods we used to engage fishermen and collect our trial fish, and eventually needed to extend the trial to include any *Kyphosus* spp. Approximately one month after doing this, POH staff had collected enough fish to begin the trial (43 in total).

During the initial weighing event, it was discovered that only 20 of the 43 fish captured for the trial remained in the cages. The pond staff believes high tide events allowed fish to escape from the cages in both treatments during their holding time due to unsecured lids on their enclosures (afterwards, they added bungee cords to secure the lids onto the cages). Thus, the experimental design had to be reduced in order to begin work with the remaining fish. Additionally, in the original experimental design, *Kappaphycus alvarezii* was to be tested, but following the El Nino event of 2016, the pond stewards were no longer able to locate this species (they find it has been wiped out locally).

With these alterations to the work plan in consideration, on January 23rd, 2017, Paepae o He'eia began an invasive algae acceptability test – feeding wild-caught *Kyphosus* spp. one of two species of invasive algae found locally (*G. salicornia,* and *A. specifiera*). Nine fish were in the *G. Salicornia* treatment, at an average starting weight of 177 ± 120 g. Ten fish began in the *A. specifiera* treatment, at an average weight of 165 ± 140 g. Daily data was asked to be collected on the water temperature, salinity, and amount of limu in the cages. The fish underwent monthly weighing events, and the trial was scheduled to conclude on June 25th, 2017.

On June 11th, the staff noted they were not able to tell whether there were fish in the cage, and on June 16th confirmed that there were no fish left in the trial. This loss was thought to have occurred due to the cage lids being left unsecured during high tide events.

# 4. Results and Discussion

## 4.1 Growth on Commercially-Available Pelleted Diets

### 4.1.1 Marine Grower Trout Diet vs. Tilapia Diet

A six-month trial of kyphosids (average start weight 386±78 g) displayed comparable growth when fed a tilapia diet (35% protein, 5% lipid) and a trout diet (45% protein, 16% lipid) (Tables 1, 2).

Table 1: Average weight gain (g) over a six-month trial of kyphosids on a tilapia diet (35% protein, 5% lipid) and a marine grower diet (45% protein, 16% lipid).

| **Treatment** | **Avg Weight Gain ± SD (g)** | **Significance** |
|---|---|---|
| Tilapia Diet | 218.22 ± 66.56 | Insignificant: p=0.909 |
| Marine Grower Diet | 220.89 ± 72.45 | |

Table 2: Average final weight (g) of kyphosids following a six-month feeding trial on a tilapia diet (35% protein, 5% lipid) and a marine grower diet (45% protein, 16% lipid).

| **Treatment** | **Avg Final Weight ± SD (g)** | **Significance** |
|---|---|---|
| Tilapia Diet | 607.22 ± 81.69 | Insignificant: p=0.264 |
| Marine Grower Diet | 642.61 ± 104.08 | |

Two methods of feeding were also tested: 1) batch feeding by hand, and 2) belt feeders to provide a steady supply of pellets throughout the day. A significantly better growth rate was observed when batch feeding was used (Table 3).

Table 3: Average daily growth (g) of kyphosids fed using batch feeding (3 times daily feeding by hand to visible satiation) vs. belt feeding regimens. Each regimen combines both diet treatments into one value for daily growth.

| Method | Daily Growth ± SD (g) | Statistical Relation | Significance |
|---|---|---|---|
| Batch | 2.10 ± .64 | A | p=0.00 |
| Belt | .85 ± .57 | B | |

Proximate and fatty acid analyses (Table 4), revealed comparable overall fat content, as well as comparable EPA and DHA fatty acids between both treatments. Significant differences ($\alpha < 0.05$) were observed in protein and ash components.

Table 4: Proximate and fatty acid analysis of wild-sourced kyphosids at an average starting weight of 386±78 g fed a tilapia diet (35% protein, 5% lipid) and a marine grower diet (45% protein, 16% lipid) over a six month growth trial.

| Metric | Avg ± SD (%) | | Significance |
|---|---|---|---|
| | **Tilapia Diet** | **Marine Grower** | |
| Protein | 66.89 ± 2.93 | 75.45 ± 2.91 | *p=0.023 |
| Fiber | 0.60 ± 0.01 | 0.75 ± 0.23 | p=0.319 |
| Ash | 3.97 ± 0.04 | 4.48 ± 0.30 | *p=0.045 |
| **Fat in Meat** | 23.83 ± 1.54 | 21.58 ± 5.75 | p=0.549 |
| Fatty Acids | | | |
| **DHA** | 0.61 ± 0.03 | 0.55 ± 0.16 | p=0.519 |
| **EPA** | 0.24 ± 0.03 | 0.24 ± 0.09 | p=0.986 |

A blind taste-test of 7 participants revealed unanimous preference for the marine grower diet treatment when served raw (the color of the flesh came out more pink (vs. grey in the tilapia diet treatment), and the taste was more positively received). Though when cooked, both treatments were of about equal preference.

Equal growth of kyphosids on tilapia and marine grower pellets has significant economic and ecological implications in terms of production, because the tilapia diet is considerably less expensive than the trout diet, and contains a greater amount of plant-derived ingredients which displace marine fish derived proteins and oils.

Although we hypothesized switching to belt feeders would increase consumption/ growth by providing consistent food availability, akin to how kyphosids graze in the wild, uneaten food was unable to be measured due to breakdown. Belt feeds were adjusted so there were "few" uneaten pellets at the time of belt reload, representing satiety but not excess. It was assumed that >90% of the feed on the belts was consumed, which is much more than the daily average consumed during the hand feeding regimen. However, data suggest this feeding method drastically reduced growth.

It is unlikely that our kyphosids reached an asymptote in their growth curve during this trial, since size records indicate significantly larger maximum body sizes are possible. Because of this, it is hypothesized that the mechanism to explain their suppressed growth rate on the belt feeding regimen may be physiological, or behavioral. It is not within the scope of this work to investigate the question any further, but we do have sufficient data to support the ongoing use of batch feeding in all subsequent trials.

Given the disparate fat content in the two tested commercial diets (trout - 45% protein: 16% lipid and tilapia - 35% protein: 5% lipid), yet comparable growth performance, overall fat, and fatty acid content between the two treatments, the data suggests kyphosids do not receive any benefit to growth performance related to fat content, and may not assimilate it into their tissue in relation to the proportion it was consumed in. However, the protein content of the fish in the trout diet treatment was significantly greater than the tilapia diet, in accordance with our expectations based on the protein:fat ratio of the two diets.

### 4.1.2 Capturing the Growth Curve

In the hatchery to market size grow-out, fish began at 2.9 ± 1.7 g and grew to an average overall weight of 479 ± 140g on the tilapia diet (TD) and 567 ± 45g on the marine grower (MG) diet (p=0.207) over 63 weeks (Figure 3). Overall growth performance was comparable between the two treatments. Average FCR over the entire trial duration was 2.9 for the tilapia treatment, and 2.6 for the marine grower treatment. Three mortalities occurred in the MG treatment (week 5, week 7, week 7), and one in the TD treatment (week 9). No further mortalities occurred throughout the 63-week trial.

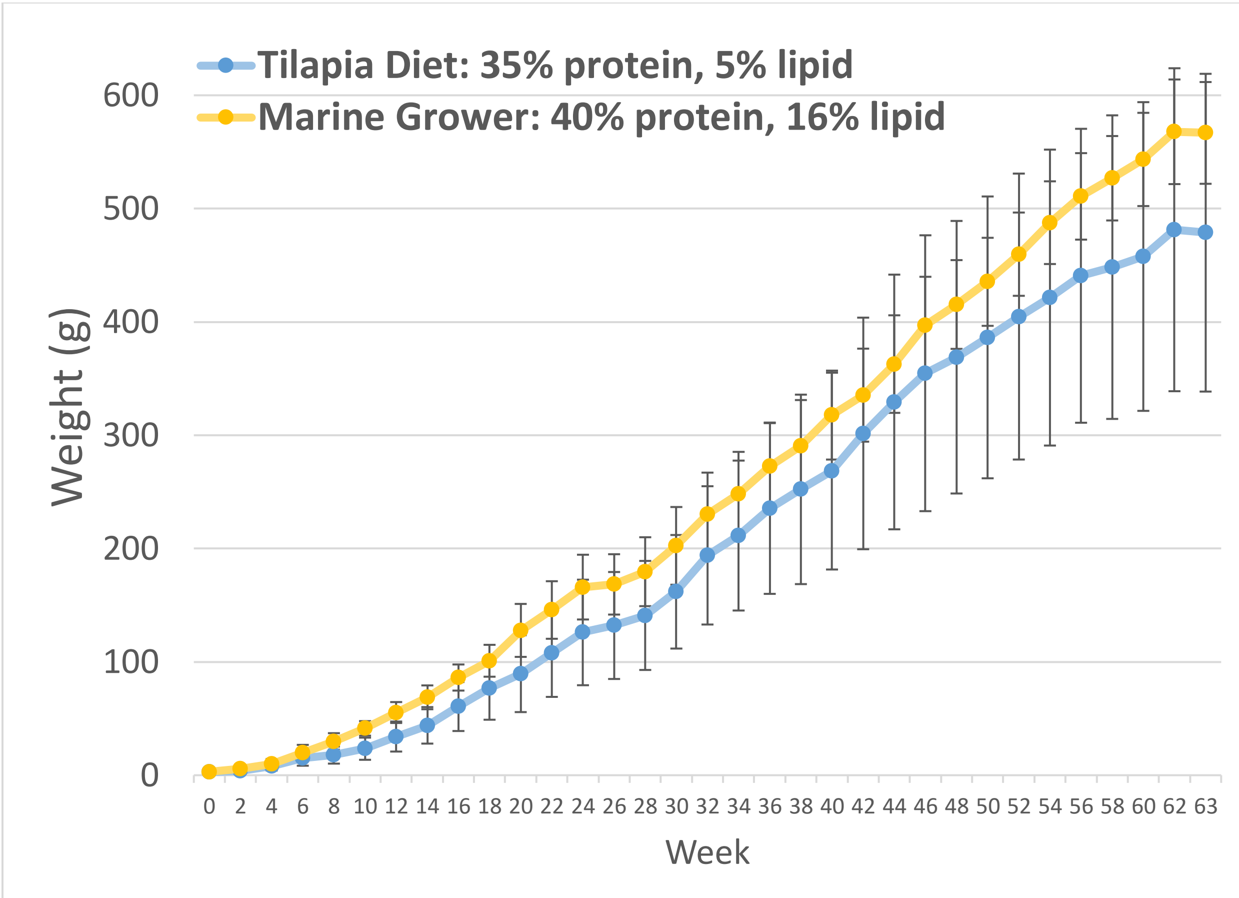


*Figure 3: Average weight (g) and standard deviation of kyphosids grown on commercially available tilapia and marine grower diets over 63 weeks (12/22/2016-3/8/18). Starting weights and n totals are: 9 fish (average 2.7 ± 1.8g) on a tilapia diet (TD), 8 fish (average 3.1 ± 1.7g) on a marine grower diet (MG).*

Viscera from three fish in each treatment were analyzed for fat content. Measurements included total body weight (bw), total visceral weight, and the weight of the fat surrounding the viscera (separated by hand). No gonads were present for measurement. From the fat measurements, a viscerosomatic index (VSI), % fat of viscera, and % visceral fat:bw ratio were calculated by diet. The MG treatment showed significantly higher fat content both within the viscera, and as a percentage of total bodyweight. There was no significant difference in overall VSI (viscera:bw); while the TD treatment had a lower visceral fat content by weight, the total visceral weight was roughly equal to MG, suggesting more mass in other organs. Overall, both diets yielded viscera >50% fat by mass (Table 5), four of six specimens showed pale and granulated livers, and no fish were spawn capable.

*Table 5:* *Visceral Fat Ratios of kyphosids fed a tilapia diet (TD: 35% protein: 5% lipid) or a marine grower diet (MG: 40% protein: 16% lipid) over a 63-week growth trial. *= p<0.05, VSI=viscerosomatic index.*

| Treatment | VSI | Significance | % fat of viscera | Significance |
|---|---|---|---|---|
| TD | 7.3 | none | 52.5 | *p=0.024 |
| MG | 8.5 | p=0.233 | 62.0 | |

Grow-out comparison of kyphosids from hatchery- to harvest-size displayed equal growth between the tilapia and marine grower treatments. This has significant economic and ecological implications in terms of production, because the tilapia diet is considerably less expensive than the trout diet, and contains a greater amount of plant-derived ingredients which displace marine fish-derived proteins and oils. The average FCR of 2.55 on commercial diets is suggestive of a nutritional inadequacy that could be improved through customization of a kyphosid-specific formulation.

## 4.2 Algae Diets for Marine Herbivorous Finfish

### 4.2.1 Wild Limu Diet

The fish began at an average weight of 379 ± 185 g and finished at a weight of 400 ± 154 g over twelve weeks, representing insignificant growth. Individual tracking (Figure 4) reveals the trend of stagnant or declining growth exists in each individual fish, so it is clear that one or two 'poor' performers were not dragging down the average growth performance of the group.

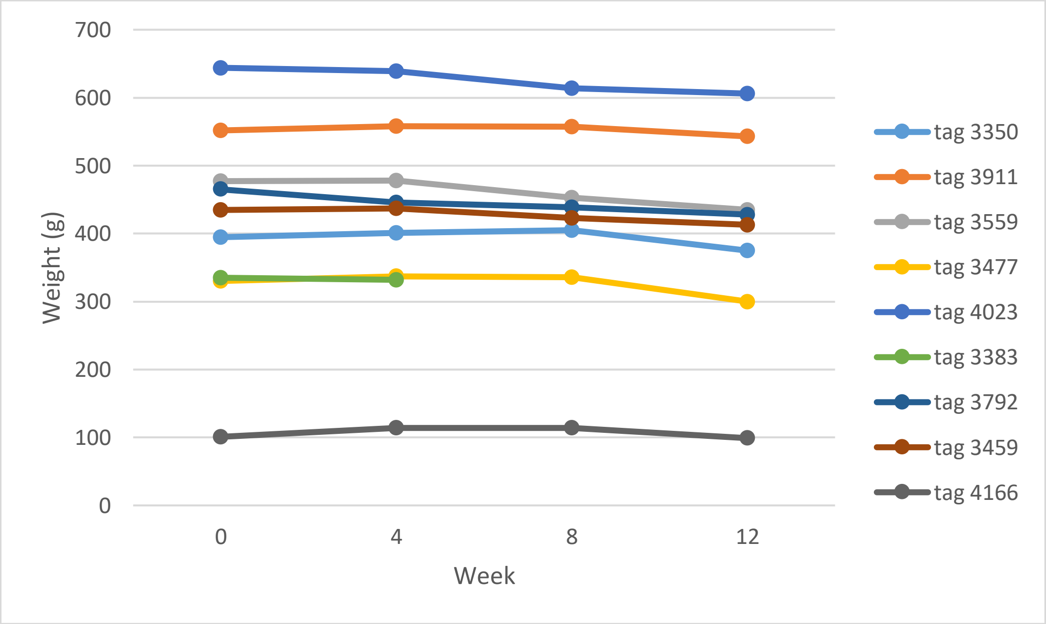


*Figure 4: Individual performance of kyphosids fed a wild macroalgae diet over a 12-week performance period.*

The stagnant growth performance of the kyphosids held on the natural limu diet was counter to our expectations, and the trial was ended after twelve weeks. The quality of the holding basin limu was monitored prior to feeding, and we don't have reason to believe the quality of the feed deteriorated over the duration of the trial. It is possible that their grazing behaviors were disrupted in the tank environment causing suppressed feeding (the algae hung vertically on the side of the tank, against a dark tank background, held in a mesh sleeve and at a different density/pattern than it would grow in the wild). Since we also observed suppressed growth when attempting to model a grazing pattern with belt feeders in the pelleted diet treatments, there could be an undercurrent of behavioral cues for grazing that have not been appropriately modelled in the tank environment. The data demonstrate that they grow appreciably on pelleted diets fed at the surface (presumably a novel feeding method for them) in batches throughout the day.

### 4.2.2 Algae-Based Pellets for Kyphosids

Growth of kyphosids on the algae pellet trial (after the rancidity issues were resolved) revealed no significant difference between the average weights of the fish at any point in the trial (Table 6). This is true both between diets and among tanks.

*Table 6: Average fish weight (g) ± Standard Deviation of kyphosids fed a tilapia diet (35% protein: 5% lipid) or an algae-based experimental pellet over a 12-week trial.*

| **Week** | **Algae Diet (AD)** | **Tilapia Diet (TD)** | **Significance** |
|---|---|---|---|
| 0 | 624 ± 98 | 595 ± 115 | p=0.474, insignificant |
| 2 | 607 ± 95 | 593 ± 114 | p=0.722, insignificant |
| 4 | 599 ± 99 | 613 ± 131 | p=0.761, insignificant |
| 8 | 582 ± 94 | 629 ± 147 | p=0.314, insignificant |
| 12 | 575 ± 95 | 637 ± 156 | p=0.203, insignificant |

At week 12, replicate tanks were consolidated into one tank per treatment to provide indication of whether consumption or growth would increase (it was hypothesized that lower density may have been inhibiting feed response). Increasing school size may increase feed consumption, however once average fish growth is overlaid, there is no correlation between the increased feed consumption and improved growth (Figure 5). This holds true for both TD and AD treatments, and so cannot be directly explained by nutritional deficiency of the AD formulation.

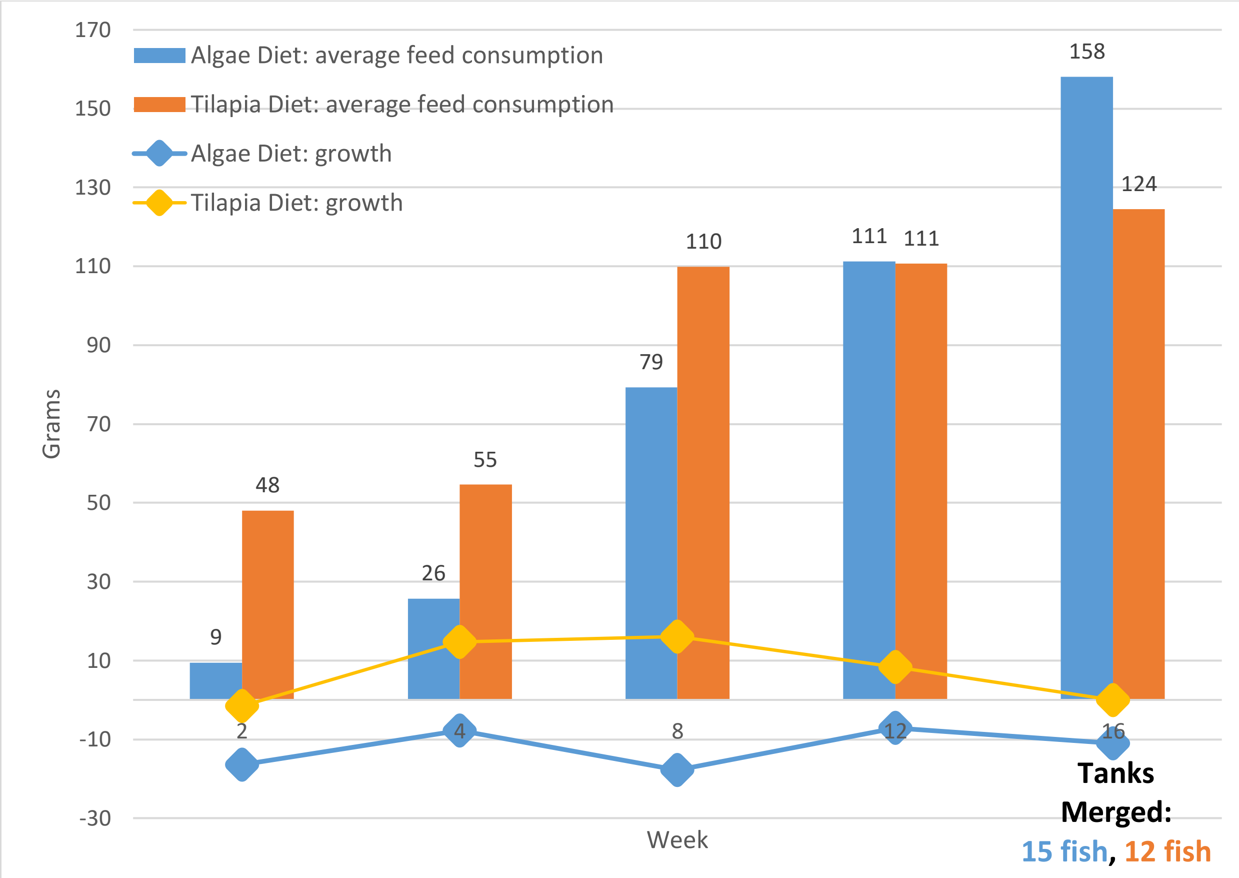


*Figure 5: Average growth and feed consumption (g) of kyphosids fed a tilapia diet (Rangen, 35% protein: 5% lipid) and an algae-based pelleted diet (formulated by Dr. Rick Barrows, composition: 54% Gracilaria, 25.7% Sargassum, 15% wheat flour, and trace vitamins and minerals, top coated in fish oil). The trial was conducted to week 12 in tanks of 4-5 fish per replicate, and the final four weeks were conducted by pooling the fish into two treatment tanks of 15 and 12 fish (Algae diet and tilapia diet, respectively). Tanks were pooled as a result of suppressed feed consumption and negative growth observed at lower stocking densities.*

The overall weight loss of kyphosids on the algae-based pellet is reflected as a loss of fat in the tissue proximate and fatty acid analysis (Table 7). The protein content of the fish on the algae-based pellet was nearly double of that on the tilapia diet treatment. All fish were held on the tilapia diet prior to the trial start, when issues of rapid rancidity were preventing its commencement. During this holding period, all of the fish likely attained a similar body composition to those on the tilapia diet (i.e. very high fat content), and when the trial began, those fish in the algae treatment rapidly loss their visceral and tissue fat stores, which resulted in an incredibly lean, protein-dominated fillet. A full analysis of the algae pellet results from Dr. Barrows is forthcoming.

Table 7: Results of the proximate and fatty acid analysis of kyphosids, standardized by moisture content, fed a tilapia diet (Tilapia Diet, 35% protein: 5% lipid) or an experimental algae-based pelleted diet over sixteen weeks.

| Metric | Avg ± SD (%) | | Significance |
|---|---|---|---|
| | Algae | Tilapia Diet | |
| Protein | 90.67 ± 5.40 | 46.94 ± 2.32 | ***p<0.001 |
| Fiber | 0.72 ± 0.04 | 0.61 ± 0.19 | p=0.398 |
| Ash | 5.21 ± 0.25 | 19.22 ± 5.45 | **p=0.011 |
| **Fat in Meat** | 6.84 ± 3.58 | 32.33 ± 6.72 | ***p=0.001 |
| Fatty Acids | | | |
| **DHA** | 0.58 ± 0.06 | 0.95 ± 0.12 | **p=0.009 |
| **EPA** | 0.10 ± 0.02 | 0.23 ± 0.06 | *p=0.02 |
| Moisture (all measures have been standardized by this value) | 71.98 ± 1.48 | 51.01 ± 1.14 | ***p<0.001 |

The fillet fat content by dry weight of the fish in the Tilapia Diet treatment (32.33 ± 6.72%, Table 7) is comparable to that of kampachi (*Seriola rivoliana*) fed fishmeal-based diets.

In terms of overall fish health in grow-out durations on the commercial pellets - during the trial, Kampachi Farms technicians noticed a scale brittleness concern in the tilapia diet trial tanks. Dr. Frederic Barrows hypothesized this may have been due to a mineral deficiency. Skin and scale samples indicated a significantly lower content of Calcium, Magnesium, and Phosphorus in the fish being fed the commercial tilapia diet (Table 8).

Table 8: Mineral analysis of kyphosid skin and scales when fed a tilapia diet (Tilapia Diet, 35% protein: 5% lipid) or an algae-based pellet (formulated by Dr. Rick Barrows, composition: 54% *Gracilaria*, 25.7% *Sargassum*, 15% wheat flour, and trace vitamins and minerals) diet over a sixteen-week trial.

| Metric | Avg ± SD (%) | | Significance |
|---|---|---|---|
| | Algae | Tilapia Diet | |
| Calcium | 3.86 ± 1.01 | 0.03 ± 0.01 | **p=0.00283 |
| Magnesium | 0.08 ± 0.03 | 0.03 ± 0.00 | *p=0.0426 |
| Phosphorus | 1.78 ± 0.37 | 0.26 ± 0.01 | **p=0.00198 |

* = p<.05, **=p<.01

Demineralized scales have significantly reduced mechanical properties, owing to the important structural interaction of mineral crystals, specifically calcium phosphate apatite crystals, with collagen (Ikoma et al., 2003). The mineral content of the skin and scales of fish in the tilapia diet treatment were significantly lower in calcium, phosphorus, and magnesium compared with the fish on the algae treatment, which would suggest that this mineral deficiency was causing the brittle scales that were observed. The mineral profile in the algae pellet was sufficient to provide healthy skin and scales in visual assessment of the algae treatment tanks.

### 4.3 Invasive algae acceptability

The growth data produced from our partnership with POH has proven uninformative. This is in large part due to the loss of fish prior to the establishment of the trial, and the subsequent loss of fish in the *G. Salicornia* treatment over the first three months of the trial (due to attrition and unaccounted losses). The resulting data from the *A. specifera* cage shows no apparent trends, as fish weights 'yo-yo' from month to month. Due to the large standard deviation of starting weight, the loss of fish from month to month skews the average weight to a point where it is unreflective of the actual individual weight data (i.e. the average weight appears to increase, due to the loss of small fish 'outliers', but the individual weight of the remaining fish does not follow the trend of average weight change).

Fat content analysis and taste testing might still have provided useful information at the conclusion of this trial. This could have informed whether or not the fish had a desirable fat content and fatty acid profile (one of our original research questions). The trial was due to be completed on June 25th, 2017, upon which the fish would have been processed for samples and taste testing. Unfortunately, due to the loss of all fish prior to the final weighing event, it was not possible to collect any of this data.

The pond readily drops in salinity during high rainfall events. The range of salinities measured at algae restocking events in the cages during the trial was 13 – 34 ppt. The overall average salinity (excluding the outlier datapoints of the short-duration high rainfall events) was 30 ppt.

## 5. Conclusions

### 5.1 Potential for Commercial Culture

Overall, commercially available diets can produce a taste and texture of kyphosids that is desirable for human consumption. Growth rates indicated that growth to approximately 500g is achievable in one year on these commercial diets (which would be better optimized through custom herbivorous diet formulations). Depending on going market price and production system, however, the FCR on these commercial diets may not be economically attractive for production. The commercial tilapia diet is approximately 70% of the cost of a marine grower diet ($1.60/kg vs. $2.33/kg), due to the lower inclusion of fishmeal and fish oils ingredients, which could potentially result in large scale savings over a commercial cohort. However, the FCR achieved in these commercial pellet growth trials is not sufficient to be economically feasible. This research supports the need for further refinement of the algae-based pellet in future growth trials to improve FCR (and, thus, economy of commercial culture), while preserving flesh taste and quality for consumers.

The average overall FCR of 2.8 on commercial diets is suggestive of a nutritional inadequacy that could be improved through customization of a kyphosid-specific formulation. Grow-out comparison of kyphosids from hatchery- to harvest-size displayed equal growth between commercially available tilapia and marine grower treatments. Significant economic and ecological implications could result in production scale, because the tilapia diet is considerably less expensive than the trout diet, and contains a greater amount of plant-derived ingredients which displace marine fish-derived proteins and oils. The high visceral fat content of fish on both diets suggests a suitable pathway for utilization of these lipids may not exist in kyphosids, which provides useful feedback for future experimental formulations.

A diet based on macroalgae ingredients has the potential to be even more cost-effective (since powdered macroalgae is a major component, which can be sourced relatively inexpensively), and even more sustainable and scalable (due to the exclusion of wild-fishery derived proteins). This work resolved critical rancidity challenges with high-ash macroalgae ingredients to conduct the first ever iteration of an algae-based feed for marine herbivores (to our knowledge). Results provide direction for further refinement of the formulation to address rancidity and tune the nutritional profile.

## 5.2 Potential for Community-Scale Culture

Kyphosids were capable of eating the invasive algae which was offered at Paepae o He’eia (*G. salicornia,* and *A. specifiera*). The fish stocked in the pond trial pens persevered through heavy rainfall events, with measured salinities as low as 13 ppt. Because of their tolerance to low salinity environments, fish pond locations could be suitable for community-scale production of species such as kyphosids. No discussion of growth rates or of the palatability of kyphosids grown on invasive algae can be undertaken due to the challenges discussed in Sections 3.3 and 4.3.

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## 7. Photos


*Figure 1: Juvenile K. vaigiensis (average weight of cohort 386±78g) after being PIT tagged and weighed.*


*Figure 2: Juvenile kyphosid (average weight of cohort 41.7±10.4g) caught offshore under floating objects, being measured for a feeding trial on a tilapia diet.*


*Figure 3: A juvenile kyhposid (start weight of cohort 2.9±1.7g) at the initial weighing event for our most recent comparative trial (tilapia diet vs. marine grower), initiated 12-22-2016*

*Figure 4: Finding a site for the first cage assembled at He'eia fish pond in August 2016*

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