NRAC FINAL REPORT

Project Title: White worm, *Enchytraeus albidus*, production and marketing for live aquaculture feed

Reporting Period: 9/01/2015- 8/31/2017

Author (Chair): Elizabeth Fairchild

Key Words: white worm, aquaculture, live feed, marine, freshwater, finfish

Funding Level Total funds allocated for this project to date.

Year One: FY 2014, $76,646
Year Two: FY 2015, $75,279

PARTICIPANTS (funded participants are indicated with an asterisk)

Principal Investigator(s):

*Dr. Elizabeth A. Fairchild: Assistant Research Professor, Department of Biological Sciences, University of New Hampshire, Durham, NH 03824; ph: 603-862-4475; fax: 603-862-3784; elizabeth.fairchild@unh.edu

Dr. Michelle L. Walsh: Marine Sciences Professor, Environmental Marine Sciences, Key West Community College, Key West, FL 33040; ph: 973-713-4530; michelle.lynn.walsh@gmail.com

*Dr. Michael Chambers: Aquaculture Specialist, NH Sea Grant Extension, University of New Hampshire, Durham, NH 03824; ph: 603-862-3394; michael.chambers@unh.edu

*Dr. Fiona Wilson: Assistant Professor of Strategy, Social Entrepreneurship, and Sustainability, Department of Management, Paul College of Business and Economics, University of New Hampshire, Durham, NH 03824; ph:603-862-3903; fax: 603-862-4468; fiona.wilson@unh.edu

*Dr. Kelly L. Cullen: Associate Professor of Resource Economics, Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824; ph:603-862-4811; kelly.cullen@unh.edu

Service Providers:

*Dr. Cem Giray: Chief Scientist and Vice-President, Kennebec River Biosciences, Richmond, ME 04357; ph: 207-737-2637; fax: 207-737-4504; cxgiray@kennebecriverbiosciences.com

*Dr. Jesse Trushenski: Assistant Professor, Center for Fisheries, Aquaculture, and Aquatic Sciences, Departments of Zoology and Animal Science, Food and Nutrition, Southern Illinois University Carbondale, Carbondale, IL 62901; ph: 618-536-7761; saluski@siu.edu

Cooperating, Non-funded Participants:

Dr. David Berlinsky, Director, Aquaculture Research Center, and Professor, Department of Biological Sciences, UNH, Durham, NH 03824; ph: 603-862-0007; fax: 603-862-3874; david.berlinsky@unh.edu

Dr. Reginald Blaylock, Assistant Director & Angelos Apeitos, Hatchery Specialist, Univ. Southern MS, Thad Cochran Aquaculture Center, Gulf Coast Research Lab, Ocean Springs, MS 39564; ph: 228-872-4568; fax: 228-872-4566; reg.blaylock@usm.edu

Dr. R. Christopher Chambers, Sandy Hook Laboratory, Northeast Fisheries Science Center, NOAA, 74 Magruder Rd, Highlands, NJ 07732; ph: 732-872-3075; fax: 732-872-3088; chris.chambers@noaa.gov

Mr. Peter Egelston, President, Smuttynose Brewery Co., 225 Heritage Ave., Portsmouth, NH 03801; ph: 603-436-4026; fax: 603-433-1247; peter@smuttynose.com

Mr. Gregory Fischer, Facilities Operation Manager, University of Wisconsin - Stevens Point Northern Aquaculture Demonstration Facility, 36445 State Hwy 13, PO Box 165, Bayfield, WI 54814; ph: 715-779-3461; Greg.Fischer@uwsp.edu

Dr. Jeri Fox, Aquaculture and Aquarium Science Program Coordinator, University of New England, Biddeford, ME 04005; ph: 207-602-2876; fax: 207-602-5956; jfox@une.edu

Dr. Søren Hansen, President, Sea & Reef Aquaculture, Franklin, ME 04634; ph: 207-422-2422; soren@seaandreef.com

Dr. Ronald Johnson, Aquaculture Program Manager, Montlake Laboratory, Northwest Fisheries Science Center, NOAA, Seattle, WA 98112; ph: 206-860-3458; Ronald.b.johnson@noaa.gov

Drs. Kevan Main, Program Manager of Marine and Freshwater Aquaculture & Carlos Yanes, Postdoctoral Researcher, Mote Aquaculture Park, Mote Marine Laboratory, Sarasota, FL 34240; ph: 941-388-4541 ext 27; fax: 941-374-5946; KMain@mote.org

Drs. Shaun Moss, Acting President and CEO for Scientific Programs & Dustin Moss, Director of Shrimp Research Program, Oceanic Institute, Waimanalo, HI 96795; ph: 808-259-7951; fax: 808-259-5971; smoss@oceanicinstitute.org

Dr. Michael Tlusty, Director of Research & Brian Nelson, Senior Aquarist, New England Aquarium, Boston, MA 02110; ph: 617-973-5200; mtlusty@neaq.org

Dr. Robert Vega, Coastal Hatcheries Program Leader, CCA Marine Development Center, Texas Parks & Wildlife, Corpus Christi, TX and Sea Center Texas, Lake Jackson, TX 78418; ph: 361-939-7784; fax: 361-939-9238; robert.vega@tpwd.state.tx.us

PROJECT OBJECTIVES

Objective 1: Measure the effects of different feeds and production lengths on white worm growth, reproductive potential, and nutritional composition.

Objective 2: Evaluate the effects of rearing container size and/or shape for white worm production.

Objective 3: Characterize and evaluate white worms as a live feed for multiple aquatic species.

Objective 4: Evaluate the nutritional composition of white worms fed different enrichment products.

Objective 5: Improve white worm production potential.

Anticipated Benefits (how the project will benefit the aquaculture industry – directly or indirectly)

This research project (1) developed "modern" white worm production protocols, which eventually could be adapted for commercial scale production, and (2) worked with many interested aquaculture sectors to identify the white worm market(s) through a series of workshops, surveys, and testing by aquaculturists, resulting in identifying the target markets and providing worms nutritionally customized for those consumers (species). This research promotes sustainable, environmentally friendly tactics in its use of recycled, local, waste by-products for worm feed, and a low carbon footprint. This research may yield economically viable techniques for those aquaculturists looking to diversify and a readily-available product for the aquaculture market.

PROGRESS ACHIEVED COMPLETING OBJECTIVES

OBJECTIVE 1: Measure the effects of different feeds and production lengths on white worm growth, reproductive potential, and nutritional composition.

White worms are notoriously unfussy when it comes to feeding protocols; worms will survive on just about anything including cooked vegetables, baby cereal, stale fish feed, hot dog buns, and coffee grinds. This diet flexibility is one of the main advantages of white worm production; however, we do not know which feed promotes the fastest growth and production. Evaluating and determining which feeding protocols (optimal feeds and culture period before harvest) is paramount to developing large-scale and cost-effective white worm production techniques.

Our aim is to produce a live feed that can easily and cheaply be incorporated into the routine of aquaculturists, thus, the following dietary experiment was framed to distinguish the most optimal, locally-sourced feeds available to most coastal communities. We examined white worm potential as "local recyclers" by conducting a common garden experiment testing five feed treatments (coffee grinds, brewery wastes, stales [old bakery products], produce, and sugar kelp grown at UNH) over the course of different production cycles (6, 9, 12 wks) during Year 1 of the project. At the end of each production cycle, the worm population and reproductive output were calculated from each replicate. In addition, to evaluate the effects of feed and production cycle length on worm nutrition, subsamples of the worms from each experimental unit (n=45) were shipped to and analyzed by Dr. Jesse Trushenski (Southern Illinois University, Carbondale) for proximate composition and fatty acid profiles, at the beginning and at the end of the experiment.

We found that feed type and production cycle duration affected white worm biomass, reproductive potential, and proximate and fatty acid composition. In general, white worm cultures fed coffee grounds, stale bread, and spent brewing grains had higher production yields than cultures fed mixed produce or sugar kelp. Dependent on feeds and production cycle duration, white worms were high in protein (49-69%) and lipids (10-27%) and low in ash (5-8%), indicating that they would meet the dietary needs of species requiring a high protein, relatively high lipid, low ash diet. Compared to fatty acid profiles reported for standard live feeds like rotifers, *Artemia*, and copepods, white worms provided less n-3 long-chain polyunsaturated fatty acid content (DHA 0-0.5%, EPA 2-18%, total LC-PUFAs 4-25%), with the highest levels in worms fed mixed produce or sugar kelp. White worms exhibit many attractive characteristics as feeds, but commercialization will require improved culture techniques to produce greater worm biomass while reducing production costs. Depending on the target species, white worms may need enrichment to increase n-3 LC-PUFA levels.

This study was published in *Aquaculture* and the paper is included with the final report.

OBJECTIVE 2: Evaluate the effects of rearing container size and/or shape for white worm production.

This objective, examining the effect of culture container shape and size on worm productivity, was not completed. During the course of the project, we realized that this experiment was much less critical to evaluating the potential for using white worms by the aquaculture industry. Instead, we focused our resources on protocols that need to be determined in order to successfully complete Objective 3 – mainly establishing shipping and receiving guidelines for our worm testers. In addition, we became acutely aware while harvesting live worms for analyses, that coming up with more efficient harvesting techniques is the key bottleneck to scalability of white worms. We diverted funding from Objective 2 to partially support a graduate student to work exclusively on Objective 5 – improving white worm production potential.

OBJECTIVE 3: Characterize and evaluate white worms as a live feed for multiple aquatic species.

White worms were characterized and evaluated as a live feed for multiple aquatic species through a series of steps.

Biosecurity concerns:
White worm diagnostic testing with Dr. Giray at Kennebec River Biosciences was completed to formulate and provide pathogen screening strategies for white worms to ensure we provide a bio-secure product for the aquaculture industry. All viral, bacterial, and parasitic assays were negative, and results were shared with worm testers and presented in a poster at Aquaculture America 2016.

Live white worm shipping and receiving protocols:
Prior to shipping live white worm samples to industry stakeholders for testing and feedback, we wanted to ensure the worms would arrive in good condition. A series of three 'test' shipments of live worms were sent from UNH to co-PI Dr. Michelle Walsh at the Florida Keys Community College (Key West, FL) in Jan. 2016. Test 1 was shipped via 2-day priority mail, took 3 days to reach its destination, and when it arrived, the water temperature was 18 °C and most worms were dead. Tests 2 and 3 were shipped simultaneously by overnight FedEx in bags injected with oxygen. The only difference between the test shipments was one sample was shipped in a semi-permeable bag and the other in a standard polyethylene fish bag. Both test samples arrived the following morning, temperature was 3 °C, but survival was higher in the polyethylene bag. From this trial and error, we determined that live white worms needed to be overnight shipped to ensure high survival upon receipt and the polyethylene bags worked better than the semi-permeable bags we tested. We then wrote up receiving guidelines for our industry stakeholders based on UNH institutional knowledge of successful white worm handling practices plus the following experiment to determine the shelf-life of white worms kept in freshwater.

Methods:
A factorial experiment was conducted twice to evaluate the effects of time (0 -14 days) and water treatment (daily water changes or no water changes) on harvested worm survival in freshwater. Each combination (water treatment x day) was replicated in triplicate (2 x 15 x 3 = 90 experimental units). Approximately 3 grams of worms were harvested from one worm culture for stocking out the experiment. Experimental units consisted of 90 25-ml beakers filled with 20 ml distilled room temperature water. Twenty (20) live white worms were added to each beaker and all beakers were placed in a refrigerator set to the middle of the temperature range. To assess the temperature experienced by the worms, one datalogger that recorded temperature hourly (Onset Computer Corporation, Bourne, MA) was placed in a beaker in the refrigerator filled with distilled water during Run 2.

Each day for up to 14 days, a total of six beakers, three from each water treatment, were removed from the fridge, allowed to "warm up" to room temperature so that the live worms would start moving, and the number of live worms were counted (Fig. 3.1). These beakers then were removed permanently from the experiment. The remaining beakers that received daily water changes were removed from the refrigerator and a dropper was used to remove the

existing water and replace it with 20 mls of refrigerated, new, distilled water. After water changes, these beakers then were returned to the refrigerator until the following day. White worm survival for each Run was analyzed by two-way ANOVA testing for the effects between water treatment and time post harvested (JMP Pro 12.2.0). All effects/differences were considered significant at $P < 0.05$.

Results:

Run 1 began on 7/26/16 and ran for 14 days; Run 2 began on 9/20/16 and ran for 10 days. Overall mean water temperature during Run 2 was 3.8 ± 0.5 °C, and remained between 3-5 °C every day.

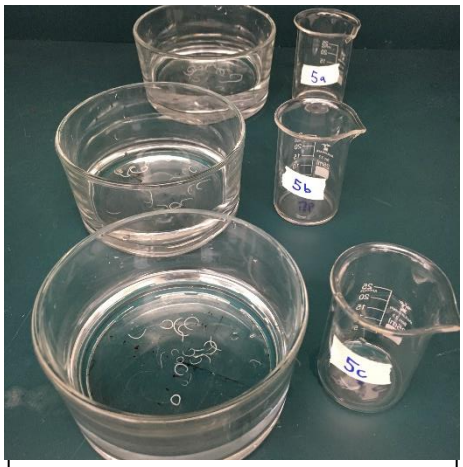


Figure 3.1. *White worms from one water treatment warming up to room temperature for daily survival assessment.*

White worm survival in both Run 1 and Run 2 was impacted by the effect of time on the water treatment (water change, no water change; $p<0.001$). Although mean worm survival began declining the day after harvest, there were no significant differences between worms that experienced water changes and those that did not until day 3 in Run 1. After day 3, survival plummeted in worms that did not receive water changes (16% survival – no water change, 93% survival – water change; $p<0.001$; Fig. 3.2). However changing water did not necessarily prolong the shelf-life of the worms. By day 4, worms from both water treatments had equally poor survival and increased mortality for worms that had been receiving water changes continued until no worms remained alive by day 6.

In Run 2, the same initial mortality pattern was observed but with worm survival decreasing quicker in the first few days; by day 1 survival had decreased to 65% and by day 4, survival was 0% in the water change treatment. For worms that did not experience water changes, survival was higher but very variable between replicates and over time (Fig. 3.2). A gradual decline in survival occurred immediately resulting in >10% mortality each day over the first 3 days. Afterwards, variability was high, usually due to one replicate dying off in entirety.

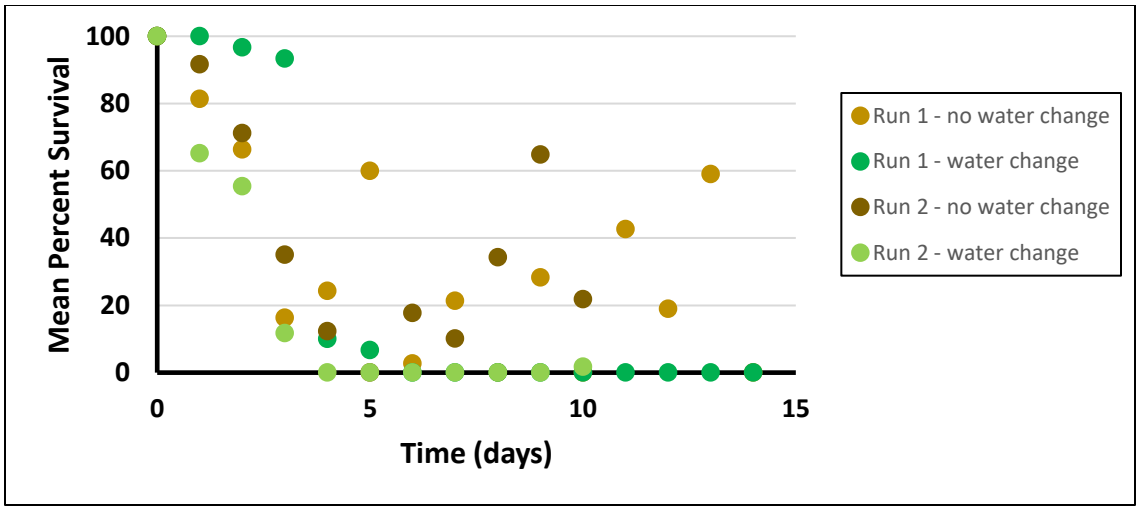


Figure 3.2. *Mean percent survival of white worms stored in refrigerated distilled water over time (error bars are not depicted). Brown circles denote vessels that did not have water changed over the course of the study (run). Green circles denote vessels in which all water was replaced daily.*

Implications:

The methods of this experiment differed somewhat from how we packaged white worms for shipment to the industry stakeholders. To get industry stakeholder feedback, approximately 7,000 worms (10 g) were harvested and gradually chilled in the refrigerator over several hours in an open pitcher of 1L distilled freshwater. Just prior to shipping, the worms and water plus another 1L chilled water were gently poured into a polyurethane fish bag, infused with oxygen, and secured in an insulated shipping box with several frozen cold packs. Samples were shipped overnight by FedEx with instructions for use and the pathogen screening results enclosed. While we tried to mimic the proportion of worm quantity to water volume of samples sent to industry stakeholders, the small 20 ml beakers used in this experiment may have created an adverse effect on worm survival in that the worms experienced colder conditions for longer periods of time relative to the worms in the larger (2L) samples. This should be evaluated to determine a more precise shelf life of the white worm samples, as well as the effects of water temperature on worm survival over time, and if longer term survival would improve with less frequent water changes. However, to err on the side of caution, we used these results to formulate our recommendations to our industry collaborators: upon receiving the white worm samples, the worms should be stored in a cool place, like a refrigerator, until use. Up to a 50% water change could be done initially if the shipment had gotten warm (>10 °C) during transport or water quality seemed poor, but otherwise was not necessary. Shelf life could not be guaranteed beyond three days so we advised our collaborators to use the white worms within 1-2 days of receiving the shipment.

Industry feedback and white worm potential:

Based on stakeholder input from the workshop held in Year 1, a survey was created for evaluating white worms as a live feed. Most original non-funded participants plus several new aquaculture stakeholders, who we connected with via word of mouth and as an outcome from giving presentations, tested live white worms in their facilities. Ten-gram samples (~7000 worms) were overnight-shipped to anyone in the US requesting worms. A total of 21 samples were shipped to 18 participants resulting in approximately 222,530 worms given out for industry feedback. Forty-one percent (41%) of participants fully completed an online survey detailing their experiences using the white worms as well as information on their facilities (e.g., species cultured, volume, live feed needs, etc.), and 47% partially completed the survey. The surveys were analyzed to determine which species or sector(s) of the aquaculture industry are most likely to benefit from using live white worms.

Survey results:

A total of 18 white worm testers either partially or fully completed the online survey. The majority of testers did not represent commercial entities but worked in an academic or research facility or in a government facility (Fig. 3.3). Several commercial facilities that we reached out to were supportive of the white worm research and interested in the results, but did not want to expose their facilities to any possible contamination from an 'unknown' product. The commercial entities that did agree to test white worms grew ornamental species only.

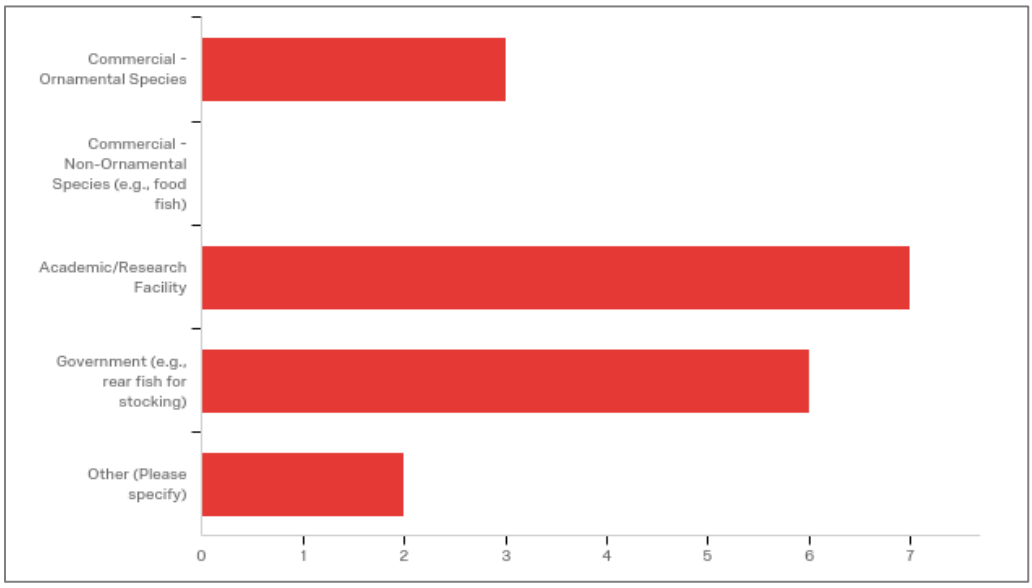


Figure 3.3. *Aquaculture sector classification of the 18 white worm testers who took the industry feedback survey. The other category includes one person in "government environmental research" and one person in international extension.*

The majority (72%) of facilities raised marine species though many also raised freshwater (39%) and brackish/euryhaline species (28%; Fig. 3.4). Almost all species (94%) were cultured in water ≥ 15 °C, with only 17% classified as cold-water species (< 15°C). These aquaculture facilities employed mostly recirculating (72%) and flow-through (50%) systems, with a few facilities (17%) using outdoor ponds too (Fig. 3.5).

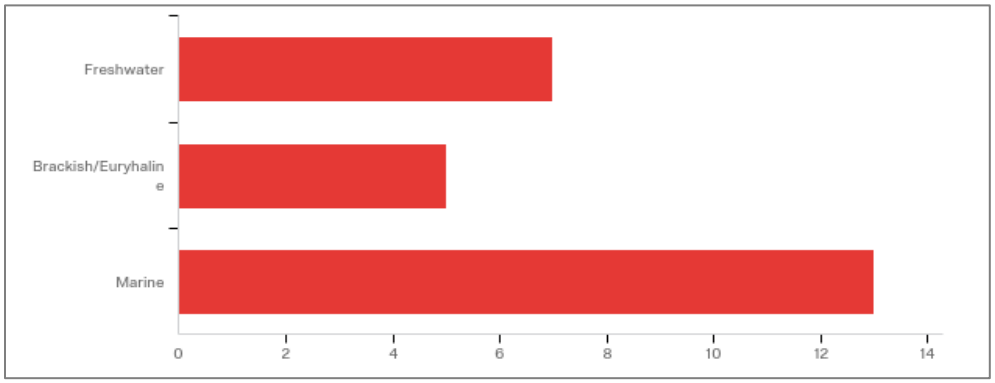


Figure 3.4. *Primary water type of facilities where white worms were tested (multiple answers possible).*

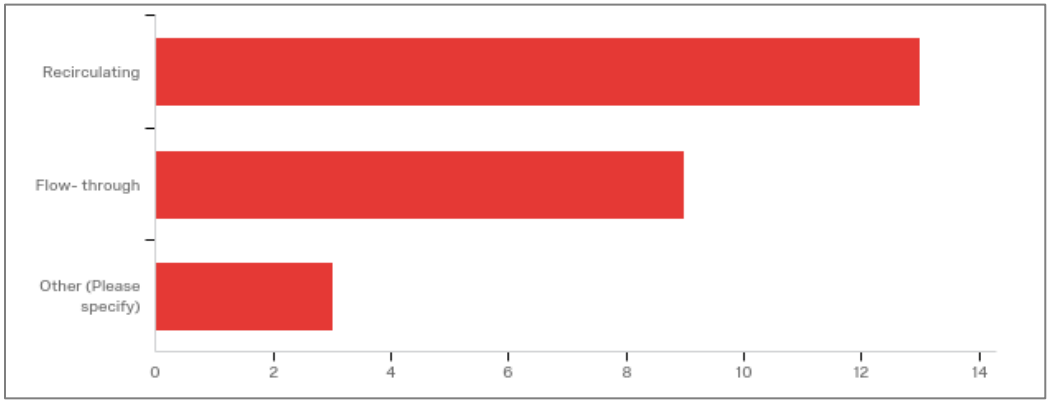


Figure 3.5. *Type of systems used in the facilities where white worms were tested (multiple answers possible).*

Of the facilities that used live feeds, 71% grew their own live feed while the remainder purchased the feed. All used rotifers and *Artemia*, 42% used copepods, and 33% used worms (blackworms, microworms, or polychaetes). Worm users grew microworms and polychaete worms in house but purchased blackworms from a supplier. White worm samples also were sent to facilities that typically don't use live feed in their normal operations; one-third (33%) of testers fell into this category.

White worms were offered to 31 marine and freshwater fish species of various life history stages, plus three bird species in a shoreline exhibit in a public aquarium (Table 3.1). The majority of fishes (58%) were ornamental fishes.

Table 3.1. *Organisms white worms were fed to and their responsiveness to the white worms when offered the first time and after multiple times, as well as the overall amount of worms eaten, compared to the organisms' standard feeds.*

	Target Species				Feeding Response Compared to Standard Feed		
	Scientific Name	Common Name	Life Stage	Size	After First Time	After Multiple Times	Total Amount Eaten
Ornamental Fishes	*Pethia conchonius*	Rosy Barb	juvenile/adult	2-3 cm	=	=	=
	Zebrasoma scopas	Scopus ang	adult	15-20 cm	↑	↑	↑
	Epalzeorhynchos frenatum	Rainbow Shark	fry	1 mm	=	=	=
	Amphiprion percula	Picasso Clownfish	adult	10-15 cm	=	=	↑
	Cirrhitidae	Hawkfish	adult	15-20 cm	=	=	↑
	Amphiprion ocellaris	Ocellaris Clownfish	juvenile/adult	1-8 cm	↑	↑	↑
	Amphiprion frenatus	Tomato Clownfish	juvenile/adult	1-8 cm	↑	↑	↑
	Premnas biaculeatus	Maroon Clownfish	juvenile/adult	1-10 cm	↑	↑	↑
	Pseudochromis fridmani	Orchid Dottyback	adult	6-8 cm	=	=	=
	Holacanthus tricolor	Rock Beauty Angelfish	adult	10-12 cm	=	=	=
	Halichoeres chrysus	Yellow Coris Wrasse	adult	6-8 cm	↑	↑	↑
	Poecilia latipinna	Sailfin Molly	adult	6-8 cm	↑	↑	↑
	Ecsenius bicolor	Bicolor Blenny	adult	6-8 cm	↑	↑	↑
	Betta spp.	Betta	adult	6-8 cm	↑	↑	↑
	Gambusia affinis	Mosquitofish	adult	6-8 cm	↑	↑	↑
	Cyprinodontiformes	Killifish	adult	6-8 cm	↑	↑	↑
	Hippocampus erectus	Lined Seahorse	adult	8-10 cm	↓	↓	↓
	Genicanthus bellus	Bellus Angelfish	adult	12 cm	=	=	↓
Other Fishes	*Acipenser fulvescens*	Lake Sturgeon	juvenile	8-13 cm	↑	↑	=
	Anoplopoma fimbria	Sablefish	early juvenile	0.5 g	↓	=	=
	Lutjanus campechanus	Northern Red Snapper	juvenile	8-13 cm	↓	=	=
	Sciaenops ocellatus	Red Drum	early juvenile	2 g	=	↑	↑
	Microgadus tomcod	Atlantic Tomcod	juvenile	8 cm	↓	↓	↓
	Sander vitreus	Walleye	fingerling	4-8 cm	↓	↓	↓
	Cyprinus carpio	Koi	adult	38-46 cm	↓	↓	↓
	Percina caprodes	Logperch	adult	10 cm	↑	=	=
	Paralichthys lethostigma	Southern Flounder	juvenile	1.5-20 cm	↓	=	=
	Oreochromis niloticus	Nile Tilapia	juvenile	3-8 cm	=	=	↑
	Menidia menidia	Atlantic Silverside	juvenile	5 cm	=	=	=
	Scaphirhynchus albus	Pallid Sturgeon	juvenile	8-13 cm	↑	↑	↓
	Pseudopleuronectes americanus	Winter Flounder	juvenile	6 cm	=	=	=
Birds	*Calidris alba*	Sanderling	adult	49-55 g	=	=	=
	Calidris minutilla	Least Sandpiper	adult	20-25 g	=	=	=
	Calidris pusilla	Semipalmated Sandpiper	adult	20-30 g	=	=	=

All target species ate the worms, however, for some species (e.g., Sablefish, Northern Red Snapper, Southern Flounder), repeated offerings of white worms were necessary to elicit a normal feeding response (Table 3.1). There were a few species (e.g.: Lined Seahorse, Atlantic Tomcod, Walleye, Koi) that did not eat as much as they typically did, even after repeated feedings of white worms. The majority of target species consumed white worms with the same intensity as they exhibit when offered their standard feeds (41%) or had a stronger feeding response to the white worms (35%). This latter group included sturgeons and ornamental fishes, specifically tang, clownfishes, wrasse, molly, blenny, betta, mosquitofish, and killifish (Table 3.1).

Most participants (56%) found using live white worms to be logistically on par with their current feed sources, or even easier to store and distribute (31%) than their standard feeds (Fig. 3.6). A small percentage (13%) found the live white worm samples to be more complicated or difficult to use compared to their current feed sources. All participants reported that there was no change in the water quality of the culture tanks from using live white worms.

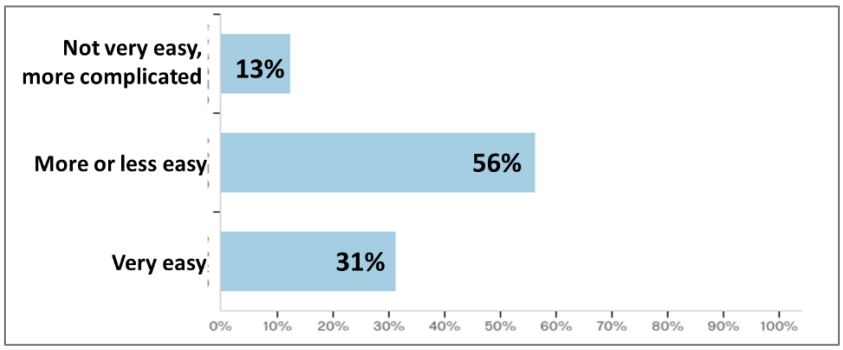


Figure 3.6. *Logistical experience of using live white worms compared to current feed sources.*

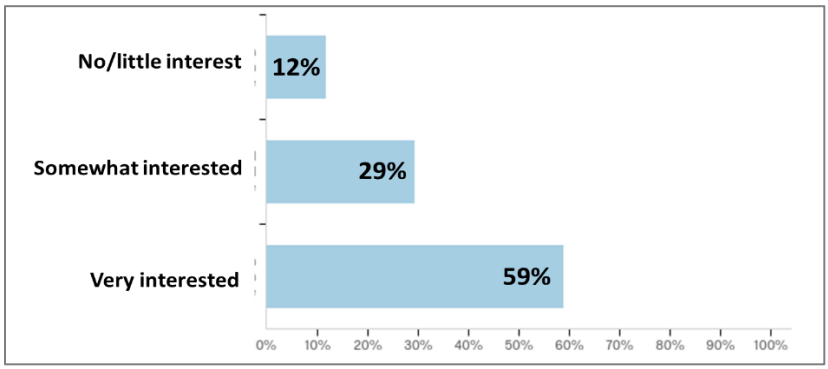


Figure 3.7. *Interest in using live white worms regularly if the product existed.*

When the participants were asked to assume if live white worms were available on a regular basis, what their likelihood of using the worms would be in their normal operations, the majority (88%) of respondents indicated that they were interested in using white worms with 59% very interested in using white worms (Fig. 3.7). Those who participated in the white worm testing, did so because they mostly were interested in finding a higher protein feed, diversifying their live feed options, and finding more suitable diets for the species being cultured (Fig. 3.8). Roughly one-third of respondents also were interested in lowering their live feed costs and utilizing diets low in fish meal and fish oil. Other reasons for participating in the white worm study included finding a live feed that would not impair water quality and finding a better live feed source for larval and juvenile fishes; aquaculturists who responded to this fed the white worms to Summer Flounder, Nile Tilapia, Sturgeon, Logperch, Rosy Barb, Rainbow Shark, Red Snapper, Walleye, Atlantic Tomcod, Atlantic Silverside, and Winter Flounder.

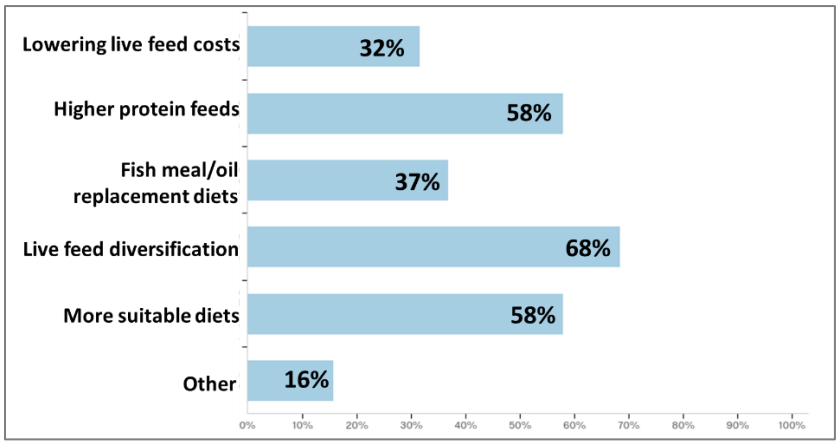


Figure 3.8. *Motivation of respondents for testing live white worms (multiple answers possible).*

Criteria that were deemed most important by the growers were condition of the white worms, ease of use, and year-round availability (Fig. 3.9). Price of the white worms and shipping frequency were rated somewhat important. Growers were less concerned with whether the worms would be certified organic and how they would be packaged. Two growers indicated in the 'other' category that how responsive the fish were to the worms and the nutritional profile of the worms were very important factors.

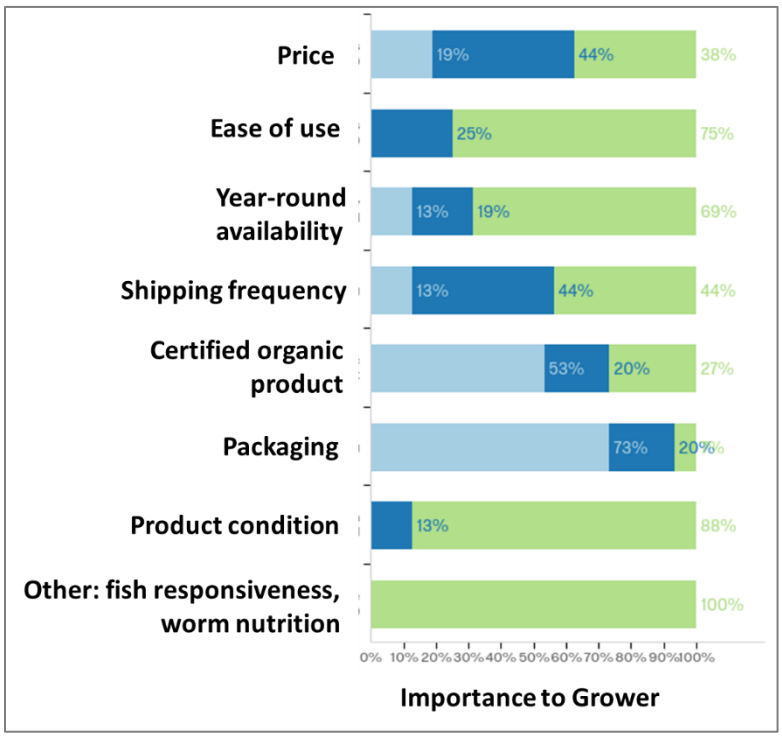


Figure 3.9. *Relative importance to growers of criteria related to live white worms. Light blue = not very important; dark blue = somewhat important; green = very important.*

Based on stakeholder input and the promising results of feeding white worms to ornamental fish, the best potential for using white worms is as a diet (live or possibly otherwise) for ornamental fishes. Ornamental culture is a growing sector in the aquaculture industry, valued at close to $30 million annually in Florida (DiMaggio, 2017). While protocols have been established to rear many of the 'typical' aquaria fishes like damsels, dottybacks, gobies, and blennies, there is a strong market demand for production of other fishes like tangs, wrasses, and butterflyfish (DiMaggio, 2017); for many of these latter species, feeding regimens have yet to be worked out. Judging from our experiences with live white worms, white worms may help with expanding the opportunities to culture these trickier species. Given that possibility, we asked the ornamental industry what they wanted nutritionally in a feed; the unanimous response was a live feed high in essential fatty acids, such as EPA and DHA.

OBJECTIVE 4: Evaluate the nutritional composition of white worms fed different enrichment products.

One of the limitations of using white worms as a feed is their relatively limited EPA and DHA content. We examined whether adding an enrichment high in fatty acids to their feed would result in white worms higher in n-3 LC-PUFAs while factoring in how cost effective these different feed strategies would be.

Does adding an enrichment to white worm feed affect the fatty acid content of the worms?

Methods:

Four white worm cultures, reared in plastic receptacles measuring 33 x 19 x 10 cm (6.4 L) and filled with sieved, seawater-dampened, organic, potting soil, were randomly chosen from the UNH stock cultures. These cultures had last been fed 3 weeks earlier. Three cultures were fed recently acquired spent brewing grains from Smuttynose Brewery (Portsmouth, NH) enriched with instant algae Reed Mariculture N-Rich High Pro Enrichment (75 mls mixed into 0.5 L grains; 1/3 cup mixture fed to each worm culture); each culture was harvested once after either 12, 24, or 48 hrs post feeding. A fourth worm culture was fed spent brewing grains only (not enriched), and was harvested 48 hrs later.

To gather sufficient sample volumes for analysis (~10 g worms), worms were harvested by placing each container on a heating pad until the worms began to congregate on the top of the soil away from the heat source. Worm aggregations were collected, transferred to seawater to remove adhering soil, drained, placed in labeled, plastic 2-dram vials, and held on dry ice until samples could be transferred to -80 °C storage. After all worm samples had been collected, they were packaged with dry ice and shipped overnight to New Jersey Feed Labs, LLC for compositional analysis.

White worms were analyzed to determine proximate and fatty acid composition. In this preliminary test, worms were not freeze dried and moisture content was not measured. Because replicate samples were

not collected in this preliminary trial, statistical analyses were not possible. However, proximate and fatty acid composition values for frozen white worms were compared to determine if any changes to the worms occurred by: 1) adding instant algae to the grains; and 2) varying the feeding duration prior to harvesting the worms.

Results:

Slight differences were observed in both proximate and fatty acid composition of white worms fed enriched and unenriched grains, and also between different feeding durations (12, 24, 48 hrs) prior to harvesting (Tables 4.1, 4.2). Maximum variation in white worm proximate composition between treatments was slight: protein = 1.52%, fat = 0.35%, and ash = 0.61% (Table 4.1).

Table 4.1. *Percent crude composition of frozen white worms after feeding on spent brewing grains for 48 hr (no enrichment), or enriched brewing grains for 12, 24, and 48 hr. Moisture content was not measured.*

	48 hr - no enrichment	12 hr	24 hr	48 hr
Protein (crude)	11.68	10.16	10.27	10.24
Fat (crude)	2.12	2.26	2.47	2.21
Ash	1.38	1.99	1.44	1.4

Variation also was observed in white worm fatty acid composition between treatments (Table 4.2). Of interest was the change in DHA from undetectable levels in worms fed only brewery grains to an increase of 1.31% of relative basis in worms fed grains enriched with instant algae and harvested 12 hr after feeding. DHA levels were present in worms fed the enriched grains with longer feeding durations too, but these levels decreased with time to 1.11% after 24 hr and 0.95% after 48 hr.

Table 4.2. *Fatty acid profile of frozen white worms after feeding on spent brewing grains for 48 hr (no enrichment), or enriched brewing grains for 12, 24, and 48 hr.*

	Duration	48 hr - no enrichment		12 hr		24 hr		48 hr	
		Relative	Sample	Relative	Sample	Relative	Sample	Relative	Sample
Fatty Acid Profile	C# : Dbl. Bonds	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %
Caprylic	8:0	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Capric	10:0	0.28	0.00	0.26	0.00	0.23	0.00	0.20	0.00
Lauric	12:0	1.19	0.02	1.02	0.01	1.06	0.02	1.07	0.02
Myristic	14:0	5.72	0.10	5.41	0.03	5.44	0.10	5.16	0.09
Myristoleic	14:1	0.95	0.02	0.79	0.00	0.64	0.01	0.75	0.01
Pentadecanoic	15:0	0.47	0.01	0.65	0.00	0.46	0.01	0.48	0.01
Palmitic	16:0	5.21	0.09	8.52	0.05	6.71	0.13	5.90	0.11
Palmitoleic	16:1	3.05	0.05	2.63	0.01	2.80	0.05	2.92	0.05
Heptadecanoic	17:0	0.35	0.01	0.48	0.00	0.38	0.01	0.28	0.01
Stearic	18:0	3.21	0.06	3.70	0.02	2.85	0.05	2.84	0.05
Oleic	18:1ω9	2.78	0.05	6.83	0.04	3.93	0.07	3.56	0.06
Oleic	18:1ω7	1.02	0.02	1.42	0.01	1.29	0.02	1.28	0.02
Linoleic	18:2ω6	25.26	0.44	25.15	0.14	27.97	0.53	24.81	0.45
Linolenic	18:3ω3	2.58	0.04	2.49	0.01	2.69	0.05	2.34	0.04
Arachidic	20:0	0.18	0.00	0.22	0.00	0.25	0.00	0.20	0.00
Eicosanoic	20:1ω11	3.30	0.06	2.00	0.01	2.82	0.05	3.03	0.05
Eicosanoic	20:1ω9	0.35	0.01	0.56	0.00	0.49	0.01	0.43	0.01
Eicosadienoic	20:2ω6	8.49	0.15	6.95	0.04	7.71	0.15	8.10	0.15
Eicosatrienoic	20:3ω6	0.85	0.01	0.60	0.00	0.56	0.01	0.51	0.01
Eicosatrienoic	20:3ω3	0.44	0.01	0.27	0.00	0.31	0.01	0.30	0.01
Arachidonic	20:4ω6	8.72	0.15	5.65	0.03	5.96	0.11	6.72	0.12
Eicosapentaenoic (EPA)	20:5ω3	2.56	0.04	2.13	0.01	2.21	0.04	2.53	0.05
Docosapentaenoic	22:5ω6	0.00	0.00	0.36	0.00	0.41	0.01	0.38	0.01
Docosapentaenoic	22:5ω3	0.33	0.01	0.36	0.00	0.33	0.01	0.28	0.01
Docosahexaenoic (DHA)	22:6ω3	0.00	0.00	1.31	0.01	1.11	0.02	0.95	0.02
Lignoceric	24:0	0.00	0.00	0.20	0.00	0.26	0.00	0.00	0.00
Other	n/a	22.49	0.39	20.04	0.11	20.93	0.40	25.00	0.45
	Total	100.00	1.73	100.00	0.56	100.00	1.90	100.00	1.80
	Total % ω3	5.91	0.10	6.57	0.04	6.85	0.13	6.39	0.11
	Total % ω6	43.31	0.75	38.70	0.22	42.61	0.81	40.51	0.73

Implications:

From this very precursory study, it appears that white worms can metabolize enrichments like instant algae which can augment their fatty acid content, and in particular, elevate their DHA levels. In addition, it is likely that these nutritional benefits wane with increasing time post-feeding.

When should white worms be harvested to maximize the effects of an enrichment?

Methods:

An additional six white worm cultures were randomly chosen from the UNH stock cultures. These cultures had last been fed 2 weeks earlier. Three cultures were fed recently acquired spent brewing grains enriched with instant algae Reed Mariculture N-Rich High Pro Enrichment (75 mls mixed into 0.5 L grains; 1/3 cup mixture fed to each worm culture); each culture was harvested once after either 4, 6, 8, 10, or 12 hr post feeding. A sixth worm culture was fed spent brewing grains only (not enriched), and was harvested 4 hr later.

Worm samples (~10 g) from each culture were harvested and stored as per prior methods. After all worm samples had been collected, they were packaged with dry ice and shipped overnight to New Jersey Feed Labs, LLC for compositional analysis.

White worms were analyzed to determine proximate and fatty acid composition. Worms were not freeze dried but moisture content was measured. Proximate and fatty acid composition values for frozen white worms were compared to determine if changes to the worms occurred by reducing the feeding duration to <12 hr prior to harvesting the worms. Because replicate samples were not collected in this preliminary trial, statistical analyses were not possible.

Results:

Similar to the first preliminary enrichment trial, differences were observed in both proximate and fatty acid composition of white worms fed enriched and unenriched grains, and also between different feeding durations (4, 6, 8, 10, 12 hr) prior to harvesting (Tables 4.3, 4.4). Maximum variation in white worm proximate composition between treatments was slight: protein = 1.05%, fat = 0.33%, and ash = 0.13% (Table 4.3). Fat content was lowest in the worms fed unenriched grains but only differed by 0.03% from those worms harvested after 12 hr feeding duration. Based on only one replicate per treatment, a potential trend may exist of increased fat content with increased enrichment duration up to 10 hr (Table 4.3) and this also is reflected in the proportion of DHA relative to other fatty acids (Table 4.4).

Table 4.3. *Percent crude composition of frozen white worms after feeding on spent brewing grains for 4 hr (no enrichment), or enriched brewing grains for 4, 6, 8, 10, and 12 hr.*

	4 hr - no enrichment	4 hr	6 hr	8 hr	10 hr	12 hr
Moisture	87.29	87.14	86.95	86.58	86.34	87.46
Protein (crude)	7.38	7.50	7.45	7.98	7.78	6.93
Fat (crude)	1.86	1.93	1.99	2.01	2.19	1.89
Ash	0.93	0.89	0.89	0.89	0.98	0.85

Table 4.4. *Fatty acid profile of frozen white worms after feeding on spent brewing grains for 4 hr (no enrichment), or enriched brewing grains for 4, 6, 8, 10, and 12 hr.*

	Duration (hrs)	4 - no enrichment		4		6		8		10		12	
		Relative	Sample	Relative	Sample	Relative	Sample	Relative	Sample	Relative	Sample	Relative	Sample
Fatty Acid Profile	C# : Dbl. Bonds	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %	Basis %
Capric	10:0	0.21	0.003	0.27	0.004	0.22	0.003	0.19	0.003	0.27	0.004	0.25	0.004
Lauric	12:0	1.11	0.015	1.30	0.018	1.21	0.017	1.14	0.016	1.27	0.020	1.42	0.022
Myristic	14:0	5.75	0.078	5.77	0.081	5.85	0.080	5.34	0.073	5.90	0.092	5.95	0.094
Myristoleic	14:1	0.75	0.010	0.78	0.011	0.83	0.011	0.76	0.010	0.80	0.013	0.78	0.012
Pentadecanoic	15:0	0.41	0.006	0.43	0.006	0.47	0.006	0.43	0.006	0.46	0.007	0.46	0.007
Palmitic	16:0	6.24	0.085	5.86	0.082	5.88	0.081	5.76	0.079	6.39	0.100	5.95	0.094
Palmitoleic	16:1	3.25	0.044	3.28	0.046	3.24	0.045	2.68	0.037	3.21	0.050	3.12	0.050
Heptadecanoic	17:0	0.25	0.003	0.25	0.004	0.32	0.004	0.21	0.003	0.32	0.005	0.35	0.006
Stearic	18:0	2.72	0.037	2.82	0.040	2.72	0.037	2.88	0.039	2.86	0.045	2.75	0.044
Oleic	18:1ω9	3.70	0.050	3.25	0.046	3.26	0.045	3.61	0.049	3.78	0.059	3.68	0.058
Oleic	18:1ω7	1.07	0.015	0.94	0.013	1.05	0.014	0.93	0.013	1.16	0.018	1.05	0.017
Linoleic	18:2ω6	26.45	0.361	25.63	0.361	26.04	0.358	26.51	0.362	25.39	0.396	26.18	0.416
Linolenic	18:3ω3	2.52	0.034	2.70	0.038	2.56	0.035	2.72	0.037	2.51	0.039	2.46	0.039
Octadecatetraenoic	18:4ω3	0.00	0.000	0.00	0.000	0.17	0.002	0.00	0.000	0.00	0.000	0.00	0.000
Arachidic	20:0	0.21	0.003	0.21	0.003	0.19	0.003	0.23	0.003	0.21	0.003	0.17	0.003
Eicosanoic	20:1ω11	3.18	0.043	2.89	0.041	2.75	0.038	2.87	0.039	2.82	0.044	2.61	0.041
Eicosanoic	20:1ω9	0.66	0.009	0.44	0.006	0.39	0.005	0.41	0.006	0.44	0.007	0.44	0.007
Eicosadienoic	20:2ω6	8.38	0.114	8.44	0.119	8.03	0.110	8.17	0.112	7.80	0.122	8.08	0.128
Eicosatrienoic	20:3ω6	0.70	0.010	0.89	0.012	0.74	0.010	0.58	0.008	0.68	0.011	0.79	0.013
Eicosatrienoic	20:3ω3	0.41	0.006	0.38	0.005	0.36	0.005	0.47	0.006	0.34	0.005	0.42	0.007
Arachidonic	20:4ω6	7.51	0.102	7.31	0.103	7.26	0.100	7.09	0.097	6.91	0.108	6.85	0.109
Eicosapentaenoic (EPA)	20:5ω3	2.31	0.031	2.38	0.033	2.47	0.034	2.75	0.038	2.58	0.040	2.41	0.038
Erucic	22:1ω11	0.34	0.005	0.30	0.004	0.32	0.004	0.25	0.003	0.32	0.005	0.32	0.005
Docosapentaenoic	22:5ω6	0.0	0.0	0.19	0.003	0.20	0.003	0.00	0.000	0.29	0.005	0.17	0.003
Docosapentaenoic	22:5ω3	0.41	0.006	0.38	0.005	0.42	0.006	0.43	0.006	0.46	0.007	0.46	0.007
Docosapentaenoic (DHA)	22:6ω3	0.00	0.000	0.69	0.010	0.77	0.011	0.71	0.010	1.06	0.017	0.84	0.013
Other	n/a	21.46	0.293	22.23	0.313	22.27	0.306	22.88	0.313	21.75	0.339	22.05	0.350
	Total	100.00	1.364	100.00	1.407	100.00	1.374	100.00	1.366	100.00	1.559	100.00	1.588
	Total % ω3	5.65	0.077	6.54	0.092	6.76	0.093	7.31	0.100	7.16	0.112	6.59	0.105
	Total % ω6	43.03	0.587	42.46	0.597	42.28	0.581	42.35	0.578	41.07	0.640	42.07	0.668

Implications:

This second preliminary trial corroborates the first trial by also showing that white worms can metabolize enrichments like the instant algae which can augment their fatty acid content, and in particular, elevate their DHA levels. Here we looked at the effects of adding an enrichment to the worm feed (brewery grains) and harvesting the worms within 12 hr after feeding. Like the first trial, it appears that these nutritional benefits are affecting by time post-feeding with worms requiring a sufficient time to feed and metabolize the enrichment. It seems DHA levels in the worms is highest when harvested after 10 hr post-feeding.

<u>Which enrichment yields the highest fatty acid content in white worms?</u>

Methods:

A common garden experiment was designed to assess the nutrient composition of white worms fed spent brewing grains enriched with various additives containing purportedly high levels of omega-3 fatty acids at the UNH Coastal Marine Laboratory. Five feed enrichments (Reed Mariculture N-Rich High Pro Enrichment [instant algae], UltraCruz Pure Salmon Oil for Dogs [salmon oil], UltraCruz Equine Pure Flax Oil [flax oil], Bob's Red Mill Premium Whole Ground Flaxseed Meal [flaxseed meal], and Bob's Red Mill

Wheat Bran [wheat bran]) plus a treatment containing no enrichment [grains] (e.g., just spent brewing grains), replicated in triplicate, were evaluated in white worm cultures held at ambient temperatures (Table 4.5). The white worm cultures were randomly chosen from the UNH stock cultures which had last been fed 1 week earlier.

Worm cultures (n=15 worm cultures) were fed recently acquired spent brewing grains enriched with one of the five enrichment treatments. For liquid enrichments (instant algae, salmon oil, and flax oil), 75 mls was mixed into 0.5 L grains. For solid enrichments (flaxseed meal and wheat bran), 0.5-1 c was mixed into 0.5 L grains or until the same consistency was reached as the liquid-enriched grains. Each worm culture was fed 1/3 c of the mixture. A sixth treatment (n=3 worm cultures) was not enriched and fed grains only. All worm cultures were harvested after 10 hrs.

Table 4.5. *Characteristics of products tested as enrichments to white worm feed (spent brewery grains) and the resulting effects to white worm EPA and DHA.*

Enrichment Treatment	Dose Amount	Worm Sample EPA* (% dry matter)	Worm Sample DHA* (% dry matter)	Shelf Life (months)	Bulk Amount	Total Doses	Total Factor Cost	Unit Cost	Average Product Cost per Unit of Increased EPA	Average Product Cost per Unit of Increased DHA
Instant algae	75 mL	0.28b	0.22a	4	0.95 L	12.7	$56.15	$4.42	$7.02	$2.55
Salmon oil	75 mL (33.5 g)	0.48a	0.23a	4-10	907 g	27.1	$21.00	$0.77	$0.75	$0.91
Flax oil	75 mL	0.20b	0.01b	12	3.8 L	50.7	$38.00	$0.75	n/a	$0.03
Flaxseed meal	113 g (1 cup)	0.23b	0.00b	6	453 g	4.0	$3.39	$0.85	n/a	$38.00
Wheat bran	55.5 g (1 cup)	0.25b	0.00b	12	227 g	4.1	$1.69	$0.41	$1.13	n/a
Grains	0 mL	0.20b	0.00b	n/a	n/a		$0	$0		

*Differing letters within a column denote significant differences ($p<0.05$) between enrichments.

Nutritional analysis:

Duplicate 10 g samples of each feed treatment and worm samples (~10 g) from each culture were harvested, snap frozen on dry ice, and stored in -80 °C storage until analyses. After all samples had been collected, they were freeze dried for 48 hr, then packaged with dry ice and shipped overnight to New Jersey Feed Labs, LLC for proximate and fatty acid composition analysis.

Mean proximate and fatty acid composition values for feeds were compared using Kruskal-Wallis followed by a Dunn Test with an adjusted p-value. White worms were compared using one-way ANOVA followed by Tukey's HSD pairwise comparison tests (R version 3.2.1.).

Although the response variables reflected data collected or calculated from many pooled worms collected from an individual culture container, replicate culture containers were considered

experimental units (N = 3) for all statistical analysis. All effects/differences were considered significant at $P < 0.05$.

Economic analysis:

To calculate the unit cost of using each enrichment product, the total factor cost (i.e., shelf price of the enrichment) was divided by the number of total doses (or bulk amount/dose amount).

The average product cost gauges which enrichment will boost these nutrients at the least cost per EPA and DHA percent composition in the final product (white worms), or, in other words, how can white worms be grown to produce the highest levels of targeted nutrients in the most cost-effective way.

To calculate average product cost of EPA concentration per 0.5 L of grain feed, the total cost of the supplement was divided by the change in percent composition of EPA:

$$TFC_{EPA} / [N_{EPA2} – N_{EPA1}]$$

where:

TFC_{EPA} is the total factor cost of the supplement per 0.5 L of grain.

N_{EPA2} is the percent concentration of EPA in white worms that were fed grains with the supplement in question.

N_{EPA1} is the percent concentration of EPA in white worms fed grains without that supplement.

These calculations were applied to each supplement and calculated for both EPA and DHA, as shown in Table 4.5.

Results:

Feeds varied as a result of the enrichment product added with protein ($p<0.001$), fat ($P<0.001$), and ash ($p<0.001$) all changing significantly (Table 4.6). Protein was highest (22-23%) in unenriched grain or grain enriched with flaxseed meal, and lowest (10%) in grains enriched with flaxseed oil and salmon oil. The inverse relationship was observed for fat content: flaxseed oil or salmon oil added to grains yielded the highest fat content at 2% compared to <1% in unenriched grain or grain + wheat bran. Ash content ranged from 1% to 9% and was highest in grain + instant algae and lowest in grain + flaxseed oil or salmon oil.

Proximate composition of white worms was mostly unaffected by feed enrichment (Table 4.6). There were no differences in either the protein ($p=0.538$) or fat ($p=0.258$) content of the worms fed any of the enriched diets. Worm composition was 55-59% protein and 15-17% fat. Only ash content ($p=0.002$) was affected by the enrichments with flaxseed meal (5.2%) and salmon oil (5.3%) having lower ash than either grain (6.3%) or flaxseed oil (6.2%), but neither grouping differing significantly from instant algae (5.7%) or wheat bran (5.6%).

Significant differences in feed fatty acid composition were detected in all fatty acids analyzed between different enrichment treatments (Table 4.6). Worm fatty acid composition also was significantly affected by feed enrichment treatment in most cases (Table 4.6). Of particular interest, worms fed grain enriched with salmon oil had the highest EPA content (sample content = 0.48%, relative content = 3.91%) compared to worms fed the other enrichments (sample content range = 0.20-0.28%; relative content = 1.84-2.47%; Table 4.6). DHA content was highest in worms fed grains enriched with either salmon oil (sample content = 0.23%, relative content = 1.83%) or instant algae (sample content = 0.22%, relative content = 1.94%), and compared to worms fed all remaining enrichment treatments enrichments (sample content range = 0-0.01%; relative content = 0-0.06%; Table 4.6).

Table 4.6. *Proximate, EPA, and DHA composition of spent brewing grain; grain enriched with flaxseed oil, flaxseed meal, salmon oil, instant algae, or wheat bran; and white worms fed these feeds. Least-square means and P-values are provided for both feed and worms. For parameters exhibiting significant enrichment treatment effects, means with different letter labels are significantly different (P < 0.05).*

			Enrichments					
Parameter	Component	Grain	Flaxseed Oil	Flaxseed Meal	Salmon oil	Instant Algae	Wheat bran	P-values
Protein (% dry matter)	Feed	21.93a	10.31c	22.63a	10.43c	20.16b	19.69b	<0.001
	Worms	55.87	55.15	58.72	56.23	55.09	55.04	0.538
Fat (% dry matter)	Feed	6.67d	55.9a	24.69b	56.7a	9.07c	5.88d	<0.001
	Worms	14.56	15.82	16.1	16.94	15.36	14.96	0.258
Ash (% dry matter)	Feed	3.31b	1.67c	3.27b	1.49c	8.75a	3.56b	<0.001
	Worms	6.3a	6.17a	5.18b	5.27b	5.68ab	5.55ab	0.002
Relative EPA (% dry matter)	Feed	0bc	0bc	0bc	8.25a	1.46b	0.40c	<0.001
	Worms	2.01b	1.84b	2.05b	3.91a	2.43b	2.47b	<0.001
Sample EPA (% dry matter)	Feed	0a	0a	0a	4.05a	0.09b	0.02b	<0.001
	Worms	0.20b	0.20b	0.23b	0.48a	0.28b	0.25b	<0.001
Relative DHA (% dry matter)	Feed	0c	0c	0c	8.19a	6.68b	0.39c	<0.001
	Worms	0b	0.06b	0b	1.83a	1.94a	0b	<0.001
Sample DHA (% dry matter)	Feed	0c	0c	0c	4.03a	0.43b	0.02c	<0.001
	Worms	0b	0.01b	0b	0.23a	0.22a	0b	<0.001

The total factor costs of the supplements per 0.5 L of grain feed varied from $1.69 for wheat bran to $56.15 for instant algae (Table 4.5). While these costs are helpful in determining which supplement is the best choice to improve the nutritional content of the worms, calculating the average product cost is key because it determines the most cost-effective method for increasing a specific nutrient. Wheat bran had the least cost per percent increase of EPA ($0.01 per % EPA), which was expected given the low price of the supplement, however it may not be an optimal supplement; the increase in EPA from wheat

bran was marginal, and it did not result in any increase in DHA. Salmon oil, on the other hand, had the second least expensive cost per % increase in EPA at $0.75 per % increase, followed by flax seed ($1.13), and instant algae ($7.02). In addition, salmon oil had the least cost per percent of DHA content. It cost $0.91 per percent increase in DHA for salmon oil, as compared to $2.55, and $38.00 for instant algae and flax oil, respectively.

Implications:

Although both flaxseed and salmon oils increased the fat content in the spent brewing grains, only the salmon oil led to greater EPA content in the worms. More importantly, salmon oil enriched grains also resulted in worms high in DHA. In addition to salmon oil, grains enriched with instant algae yielded worms with equally high DHA content. However, we recommend using salmon oil over instant algae as a more cost-effective enrichment because:

1) Salmon oil has a longer shelf life if refrigerated (up to 10 months) as opposed to instant algae which must be refrigerated but only lasts 4 months, and
2) Salmon oil also costs less per percent of combined increase in EPA ($0.75) and DHA ($0.91). This is the least costly method we tested to achieve increases in these fats (Table 4.5).

How much enrichment should be added to the feed?

Based on the previous experiment, salmon oil was chosen as the most cost-effective enrichment in terms of lower price, longer shelf life, and resultant high levels of DHA and EPA in the white worms compared to the other enrichments considered. To determine if varying the amount of salmon oil added to grains would affect white worm composition, an experiment evaluating three dosage levels (low, medium, high) was conducted.

Methods:

Nine white worm cultures (3 dosage treatments x 3 replicates) were randomly chosen from the UNH stock cultures which had last been fed 1 week earlier. Worm cultures were fed 3/4 c of a blend of recently acquired spent brewing grains (0.5 L) enriched with one of the three amounts of salmon oil: 75 mls [low], 150 [medium], 225 mls [high]. All worm cultures were harvested after 12 hr.

Nutritional analysis:

Triplicate samples of each feed treatment and worm samples (~10 g) from each culture were harvested, snap frozen on dry ice, and stored in -80 °C storage until analyses. After all samples had been collected, they were freeze dried for 48 hr, then packaged with dry ice and shipped overnight to New Jersey Feed Labs, LLC for proximate and fatty acid composition analysis.

Mean proximate and fatty acid composition values for both feeds and white worms were compared using one-way ANOVA followed by Tukey's HSD pairwise comparison tests (R version 3.2.1).

Although the response variables reflected data collected or calculated from many pooled worms collected from an individual culture container, replicate culture containers were considered experimental units (N = 3) for all statistical analysis. All effects/differences were considered significant at $P < 0.05$.

Economic analysis:

To calculate the unit cost of using each enrichment product, the total factor cost (i.e., shelf price of the enrichment) was divided by the number of total doses (or bulk amount/dose amount).

The average product cost gauges which enrichment will boost these nutrients at the least cost per EPA and DHA percent composition in the final product (white worms), or, in other words, how can white worms be grown to produce the highest levels of targeted nutrients in the most cost-effective way.

To calculate average product cost of EPA concentration per 0.5 L of grain feed, the total cost of the supplement was divided by the change in percent composition of EPA:

$$TFC_{EPA} / [N_{EPA2} – N_{EPA1}]$$

where:

TFC_{EPA} is the total factor cost of the supplement per 0.5 L of grain.

N_{EPA2} is the percent concentration of EPA in white worms that were fed grains with the supplement in question.

N_{EPA1} is the percent concentration of EPA in white worms fed grains without that supplement.

These calculations were applied to each supplement and calculated for both EPA and DHA, as shown in Table 4.8.

Results:

The amount of salmon oil added to the grains affected the proximate composition of the feed ($p<0.001$; Table 4.7). Both protein and ash decreased with increasing salmon oil dosage, while the opposite trend occurred with fat.

Table 4.7. *Proximate and fatty acid composition (% fatty acid methyl esters[FAMEs]) of white worms and feeds enriched with low, medium, and high salmon oil doses. Only fatty acids representing >1% of total FAMEs in at least one treatment group or those of special interest are reported individually. Least-square means and P-values are provided for both feed and worms. For parameters exhibiting significant salmon oil dosage treatment effects, means with different letter labels are significantly different (P < 0.05).*

		Enrichment Dosage			
Parameter	Component	Low	Medium	High	P-value
Protein (% dry matter)	Feed	12.95a	10.27b	7.63c	<0.001
	Worms	57.77a	55.39ab	53.37b	0.05
Fat (% dry matter)	Feed	48.38c	61.89b	70.47a	<0.001
	Worms	18.07	19.94	22.67	0.085
Ash (% dry matter)	Feed	1.81a	1.27b	0.98b	<0.001
	Worms	5.51	6.69	5.75	0.22
Relative EPA (% dry matter)	Feed	8.1	8.28	8.33	0.35

	Worms	3.10b	3.84ab	4.66a	0.044
Sample EPA (% dry matter)	Feed	3.54c	4.62b	5.24a	<0.001
	Worms	0.40b	0.58ab	0.83a	0.037
Relative DHA (% dry matter)	Feed	7.99b	8.19ab	8.37a	0.043
	Worms	1.35b	2.38ab	3.46a	0.027
Sample DHA (% dry matter)	Feed	3.49c	4.57b	5.26a	<0.001
	Worms	0.17b	0.37ab	0.61a	0.024
Relative Myristic 14:0	Feed	0.39b	4.84a	4.89a	<0.001
	Worm	5.38	5.73	5.86	0.628
Sample Myristic 14:0	Feed	0.17c	2.70b	3.08a	<0.001
	Worm	0.7	0.86	1.04	0.13
Relative Palmitic 16:0	Feed	14.24a	14.01b	13.91b	0.001
	Worm	6.87	7.5	7.89	0.216
Sample Palmitic 16:0	Feed	6.22c	7.81b	8.76a	<0.001
	Worm	0.89	1.13	1.4	0.076
Relative Stearic 18:0	Feed	2.32	2.38	2.39	0.093
	Worm	2.67a	2.58ab	2.43b	0.049
Sample Stearic 18:0	Feed	1.01c	1.33b	1.51a	<0.001
	Worm	0.35	0.38	0.43	0.093
Relative Myristoleic 14:1	Feed	0.39	0.37	0.37	0.696
	Worm	0.7	0.59	0.55	0.063
Sample Myristoleic 14:1	Feed	0.17b	0.21ab	0.23a	0.02
	Worm	0.09	0.09	0.1	0.62
Relative Palmitoleic 16:1	Feed	5.57b	5.81a	5.85a	0.002
	Worm	3.27	3.85	4.2	0.97
Sample Palmitoleic 16:1	Feed	2.43c	3.24b	3.68a	<0.001
	Worm	0.43	0.58	0.74	0.655
Relative Oleic 18:1W9	Feed	13.91b	14.06ab	14.18a	0.024
	Worm	4.82b	6.03ab	7.19a	0.035
Sample Oleic 18:1W9	Feed	6.07c	7.85b	8.92a	<0.001
	Worm	0.63b	0.92ab	1.28a	0.039
Relative Oleic 18:1W7	Feed	3.18b	3.28ab	3.31a	0.028
	Worm	1.43	1.7	1.83	0.071
Sample Oleic 18:1W7	Feed	1.39c	1.83b	2.08a	<0.001
	Worm	0.19b	0.26ab	0.33a	0.058
Relative Eicosanoic 20:1W9	Feed	2.88b	2.96b	3.06a	0.002
	Worm	0.77b	1.08ab	1.28a	0.046
Sample Eicosanoic 20:1W9	Feed	1.26c	1.65b	1.93a	<0.001
	Worm	0.10b	0.16ab	0.23a	0.036
Relative Eicosanoic 20:3W6	Feed	0.12	0.15	0.13	0.25
	Worm	0.48	0.51	0.4	0.497
Sample Eicosanoic 20:3W6	Feed	0.05b	0.08a	0.06a	0.005
	Worm	0.06	0.07	0.07	0.515

Relative Erucic 22:1W11	Feed	7.97b	8.42a	8.64a	0.009
	Worm	1.25b	2.13ab	2.95a	0.027
Sample Erucic 22:1W11	Feed	3.48c	4.70b	5.44a	<0.001
	Worm	0.16b	0.33ab	0.52a	0.02
Relative Linoleic 18:2W6	Feed	5.40a	3.73b	2.92c	<0.001
	Worm	23.11	22.79	21.04	0.672
Sample Linoleic 18:2W6	Feed	2.36a	2.07b	1.84c	0.001
	Worm	3.01	3.34	3.71	0.193
Relative Linoleic 18:3W3	Feed	1.30a	1.12b	1.08b	0.005
	Worm	4.45	2.38	2.3	0.341
Sample Linoleic 18:3W3	Feed	0.57c	0.62b	0.68a	0.001
	Worm	0.58	0.35	0.41	0.527
Relative Arachidonic 20:4W6	Feed	0.56	0.54	0.56	0.492
	Worm	5.55a	4.62ab	3.75b	0.025
Sample Arachidonic 20:4W6	Feed	0.25c	0.30b	0.35a	0.001
	Worm	0.72a	0.67b	0.66b	0.014
Relative Docosapentaenoic 22:5W3	Feed	0.40b	1.79a	0.45b	<0.001
	Worm	0.64b	0.84ab	0.96a	0.018
Sample Docosapentaenoic 22:5W3	Feed	0.17c	0.10a	0.28b	<0.001
	Worm	0.35	0.17	0.13	0.594

Proximate composition of white worms was mostly unaffected by enrichment concentration (Table 4.7). There were no differences in either the fat (mean: 18-23% dry matter; p=0.085) or ash (mean: 5.5-6.7% dry matter; p=0.22) content of the worms fed any of the salmon oil dosages. Only the protein content (p=0.05) was affected by the enrichment treatment with protein content decreasing with increasing salmon oil dosage (means: low=58%, medium=55%, high=53% dry matter), though the medium dosage worms did not statistically vary from either the low or high dosage worms (Table 4.7).

Feed fatty acid composition was strongly affected by salmon oil dosage. All 16 fatty acids analyzed exhibited differences in the feeds due to the enrichment amount (Table 4.7). In most cases, increasing salmon oil dosage yielded higher amounts of the fatty acid. On the other hand, only half (n=8) of the fatty acids analyzed in the worms were significantly affected by the enrichment dosage: EPA, DHA, 18:0, 18:1w9, 20:1w9, 22:1w11, 20:4w6, and 22:5w3 (Table 4.7). In all cases except for stearic (18:0) and arachidonic (20:4w6) acids, worm fatty acid concentration varied significantly as follows: low dosage ≤ medium dosage ≤ high dosage.

The total factor costs of adding salmon oil per 0.5 L of grain feed varied from $0.77 for a low dosage to $2.33 for a high dosage (Table 4.8). While these costs are helpful in determining which dosage of salmon oil is the best choice to improve the nutritional content of the worms, calculating the average product cost is key because it determines the most cost-effective method for increasing a specific nutrient. In contrast to total factor costs, a high dosage of salmon oil had the least cost per percent increase of EPA ($0.33 per % EPA) compared to the low dosage ($1.05 per % EPA). Similarly, the high dosage resulted in the least cost percent increase of DHA ($0.34 per % DHA).

Table 4.8. *Nutritional and economic impact of using varying amounts of salmon oil as an enrichment to spent brewing grains for white worm feed.*

Salmon Oil Dosage	Dose Amount	Worm Sample EPA* (% dry matter)	Worm Sample DHA* (% dry matter	Shelf Life (months)	Bulk Amount	Total Doses	Total Factor Cost	Unit Cost	Average Product Cost per Unit of Increased EPA	Average Product Cost per Unit of Increased DHA
Low	75 mL (33.5 g)	0.40b	0.17b	4-10	907 g	27.1	$21.00	$0.77	$1.05	$1.24
Medium	150 mL (67 g)	0.58ab	0.37ab	4-10	907 g	13.5	$21.00	$1.56	$0.55	$0.57
High	225 mL (100.5 g)	0.83a	0.61a	4-10	907 g	9.0	$21.00	$2.33	$0.33	$0.34
None	0 mL (0 g)	0.2	0.0	n/a	n/a		$0	$0		

*Differing letters within a column denote significant differences ($p<0.05$) between dosages.

Implications:

When factoring in the effect of the three salmon oil dosages on the fatty acid composition of the white worms, and in particular the amount of EPA and DHA, a high dosage of salmon oil is the most cost-effective enrichment we tested (Table 4.8). Administering higher doses of salmon oil resulted in the largest increased of both EPA and DHA, further reducing the cost per percent of EPA and DHA concentrations found in Table 4.5. The cost per increase in EPA was reduced to $0.33 per 0.5 L of grain feed. The cost of percent DHA in worms per 0.5 L grain feed was $0.34.

Overall Conclusions

Upon fish culturists' interests in finding an alternate live feed high in high in essential fatty acids, such as EPA and DHA, we investigated whether we could alter the fatty acid content of live white worms through dietary supplements. We evaluated the effects of five different easily available supplements added to standard white worm feed (spent brewing grains). The supplements included instant algae, salmon oil, flax oil, flaxseed meal, and wheat bran.

Because the costs of the supplements varied, it was useful to calculate the average product cost to see how much each supplement cost per unit increase of nutrient concentration in white worms. Wheat bran was the least expensive way to increase EPA levels, but the increase was marginal and supplementing with wheat bran did not increase DHA in our sample. Salmon oil was the most cost-effective means of increasing DHA, and the second-most effective way to increase EPA. The combined results make salmon oil the most ideal supplement out of the ones tested. Our results also show that a high dose of salmon oil fed to white worms shortly before harvest is the most efficient means of increasing EPA and DHA levels and the nutritional value of white worms.

OBJECTIVE 5: Improve white worm production potential.

The main bottleneck in scaling up white worm production is our current harvesting system. Currently, worms are harvested by a very rudimentary heating process whereby the worm culture containers are heated from below by electric heating pads, waiting for several hours for the soil to reach a high enough temperature that causes the worms to move to the soil surface, and then gently and carefully removed by hand aggregated worms and transferring them into clean vessels with forceps. This process can take hours to harvest relatively small amounts of worms, and may adversely affect the unharvested juvenile worms and cocoons if the soil remains too hot for too long. This process is slow, inefficient, and laborious. In addition, with this process, it is not possible to harvest a worm culture completely so determining total worm biomass/culture can only be estimated. Because harvesting the worms effectively is so critical to the success of a white worm aquaculture project, I teamed up with UNH engineers Drs. Ken Baldwin and Barbaros Celikkol, and together, we are mentoring Andrew Pompeo, an Ocean Engineering Master's student. At this point in time, Andrew has completed his harvesting experiments and is in the process of writing his thesis (expected graduation of 12/17).

<u>Heat transfer in soil:</u>
Before designing a harvesting system, we had to understand how heat transferred through the soil. In particular, we were concerned with how deep the soil could be to efficiently harvest worms, while keeping the temperature in the soil from rising to a temperature that would harm the larvae and eggs. White worm eggs can withstand a temperature of 30 °C for 30 min before they begin to die (Ivleva 1973). At 25 °C, worms will start to migrate away from the heat source. Our goal was to have the soil reach a minimum of 25 °C and maximum of 30 °C. To figure this out, Andrew built a model of a guarded hot plate in SolidWorks software and used the Thermal Analysis Simulation to model the temperature at different locations in the soil. The soil depth was set to 10 cm deep.

The results from the SolidWorks model indicated that a much thinner layer of soil – 4 cm - would be necessary to reach a harvesting temperature. Different thermal conductivity values were used, but testing with actual soil was needed to understand exactly how the heat travels through the soil (i.e., worm cultures). Andrew designed an experiment and recorded the soil temperature with 6 temperature probes at 2 cm depth intervals from the surface to 10 cm deep of soil where a heat source (heating pad) was located (Fig. 5.1).

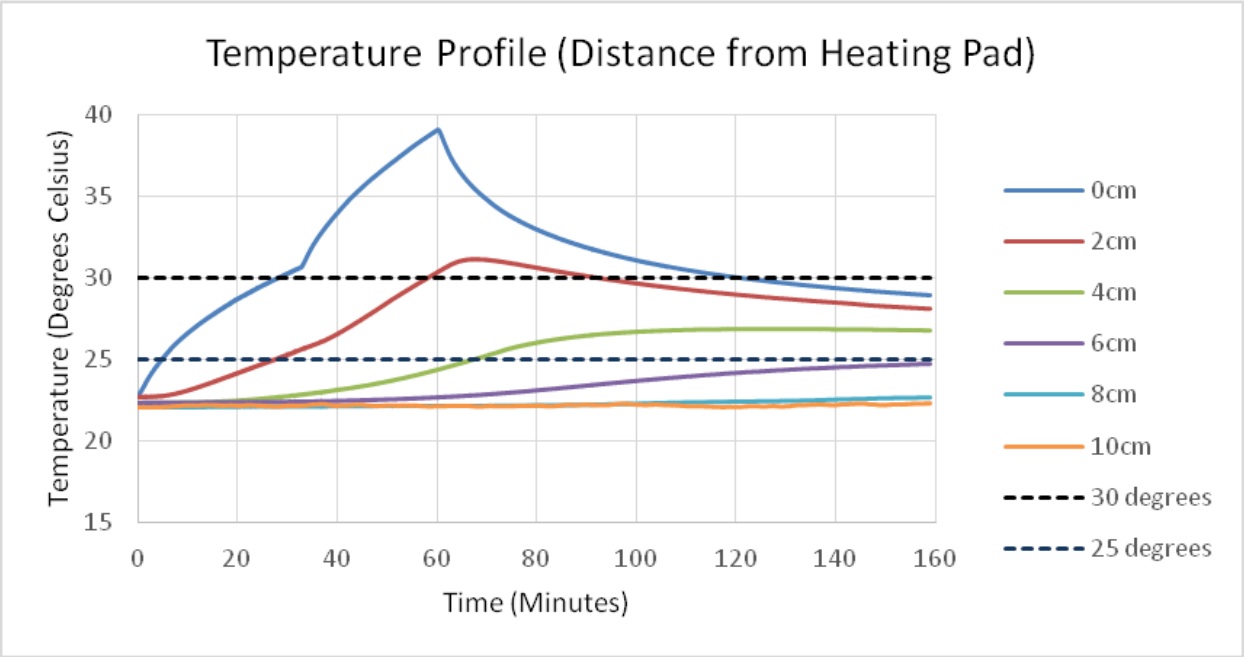


Figure 5.1. *Temperature versus time measured by each temperature probe at each 2cm interval in the soil. The temperature probe laid directly on top of the heating pad is represented by 0cm; 10cm represents the temperature probe exposed to the surface.*

Soil layers 0-4 cm reached at least 25 °C, which means, theoretically, these are the layers from which worms will migrate. Soil layers 6-10 cm will collect worms as the temperature in these layers does not exceed 25 °C. Based on these results, a similar soil experiment containing only 4 cm of soil was conducted, with three probes measuring temperature on the surface of the heating pad (beneath 4 cm soil) and three temperature probes at the surface (on top of the soil; Fig. 5.2). Based on the 4 cm depth temperature study (Fig. 5.2), we decided that a 2 cm layer of soil would be better suited for harvesting since the temperature of soil 4 cm away from the heating pad did not exceed 25 °C.

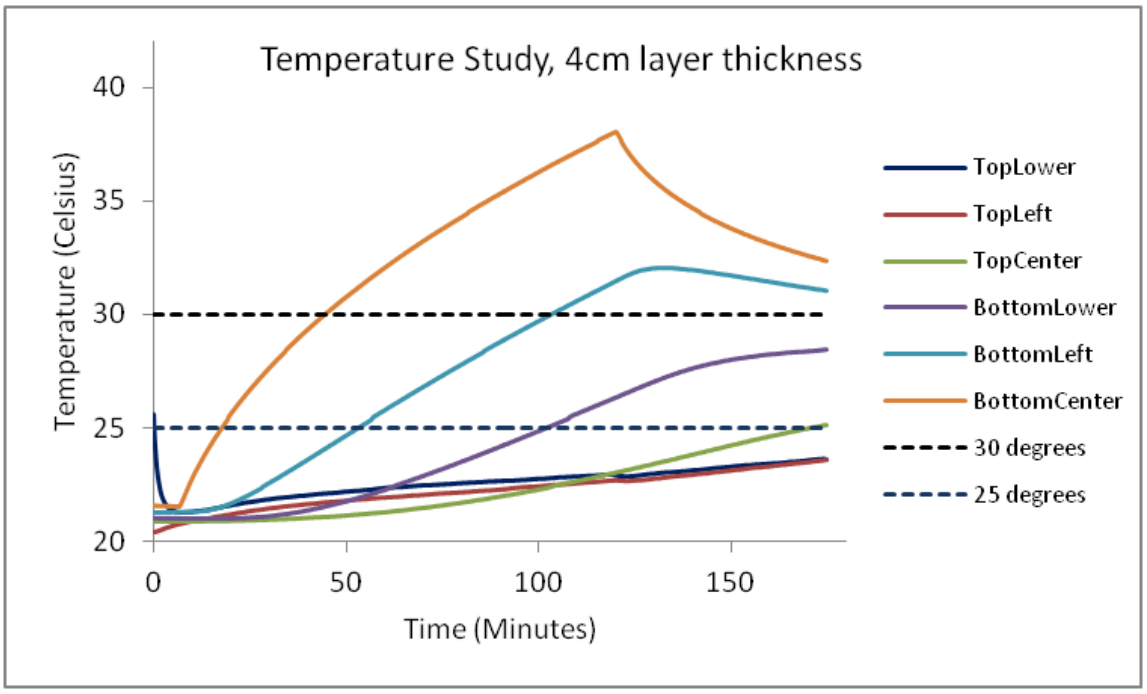


Figure 5.2. *Temperature versus time measured by three temperature probes at the surface and bottom of 4 cm of soil. The bottom temperature probes were laid directly on top of the heating pad; the top temperature probes were exposed to the surface.*

Harvester design and construction:

The next steps we took were the design, construction, and testing of a prototype harvester. We chose from three previously proposed designs. The premise of the design we selected projected heat (heating pad) downward through the soil, forcing the worms out of the soil and through a 2.5 mm screen at the bottom to a "clean" area (Fig. 5.3). The soil was contained by wooden sidewalls that also acted as insulation to retain the heat (Foam insulation and an aluminum sheet were added as extra modifications to retain heat.). A weight, placed on top of the heating pad, provided a seal to prevent heat loss. A Plexiglas sheet was placed below the harvester to collect the fallen worms.

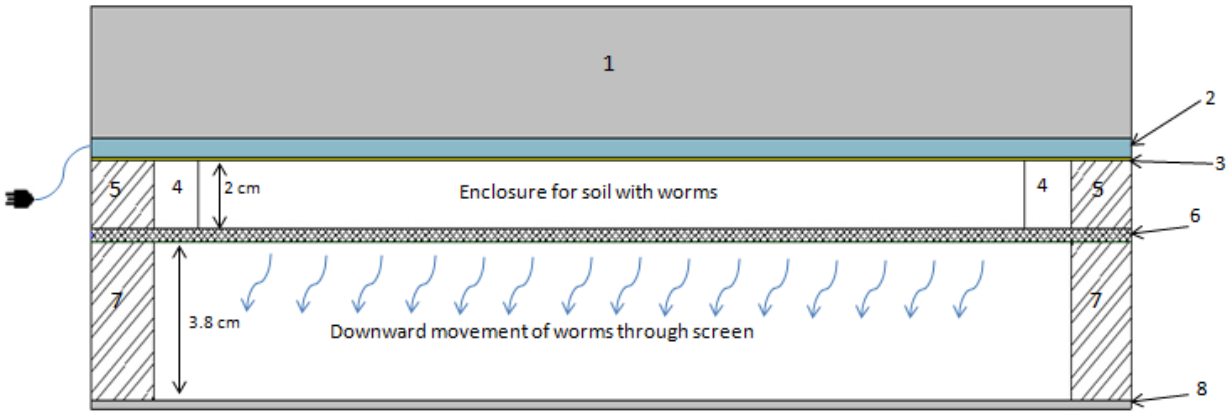


Figure 5.3. *Cross section of prototype harvester. The numbered labels correlate to different components of the harvester. 1- Weight, 2- Heating Pad, 3- Aluminum Sheet, 4- Insulation, 5- Wooden Side Walls, 6- Screen, 7- Wooden Supports, 8- Plexiglass sheet.*

A series of screen sizes (0.5 mm, 2.5 mm, and 3.2 mm) was evaluated to determine the proper screen size to minimize soil but allow worms to pass through the screen. Sieves of the varying screen sizes were used as experimental harvesters. Worms and 5 cm depth of soil were added into the sieve, and a heating pad was placed on top of the soil. The heating pad was turned on and reset every hour for three hours. At the end of three hours, observations were made on the quantity of worms that were tangled in the screen versus moved through the screen, and the quantity of soil that fell through the screen. Using a 0.5 mm screen resulted in more worms getting tangled in the screen and sometimes dying from the heat compared to the larger screens (Fig. 5.4). The 3.2 mm screen allowed more soil to fall through compared to the 2.5 mm screen (Fig. 5.4). Although the 2.5 mm screen had some worm tangling in the screen mesh and trace amounts of soil passing through the screen (Fig. 5.4), it was rated the best of the three.

Figure 5.4. *From left to right: 0.5 mm screen with high worm entanglement after 3 hrs harvesting; 2.5 mm screen with less entanglement than the 0.5 mm screen; 2.5 mm screen allowed trace amounts of soil to pass through; the amount of soil that fell through the 3.2 mm screen just after loading the sieve with soil.*

Harvesting Experiments:

A series of trials evaluating the prototype harvester was conducted in which the soil temperature and quantity of worms harvested over time were measured for each of the three heating pad power settings (Fig. 5.5). Trials for each power setting were replicated seven times. To measure the efficiency of each harvest, a known amount of worms was put into sterile soil (containing no worms) prior to the harvesting trial and harvested worms were measured at 45 min intervals. Replicate trials were averaged together to yield a mean harvesting efficiency.

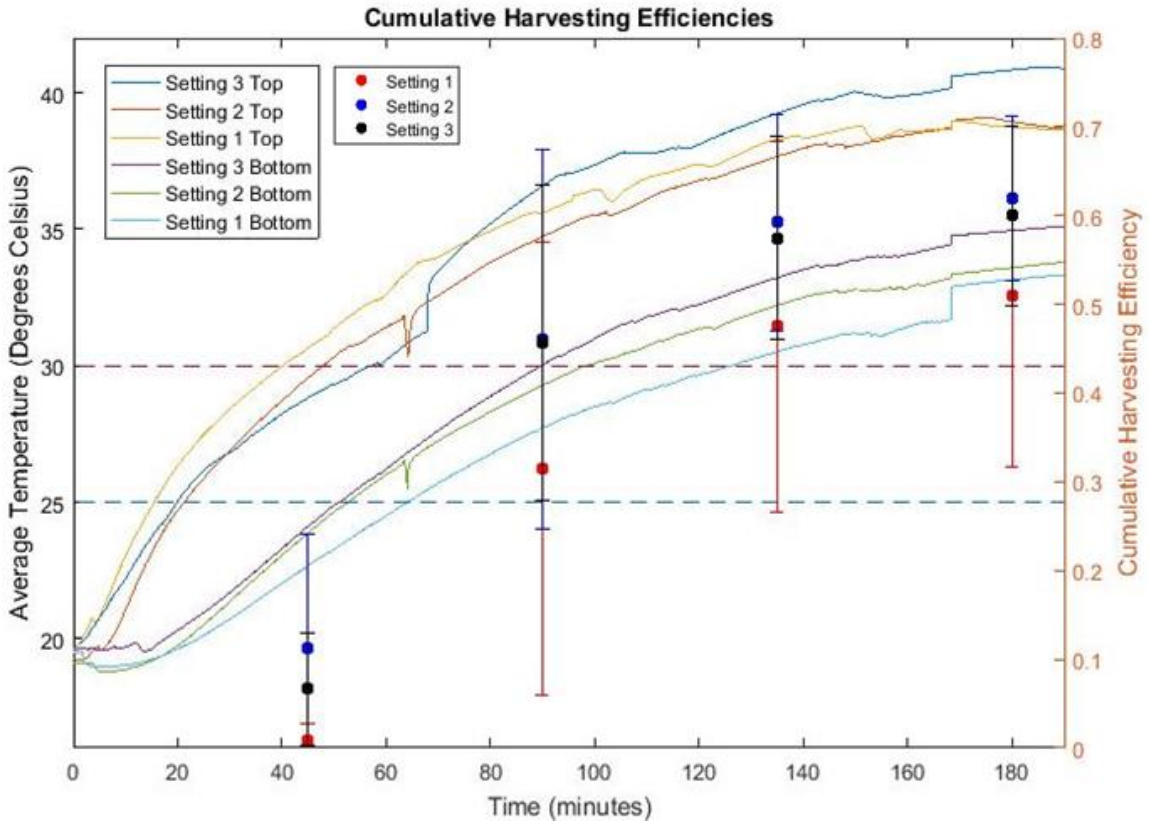


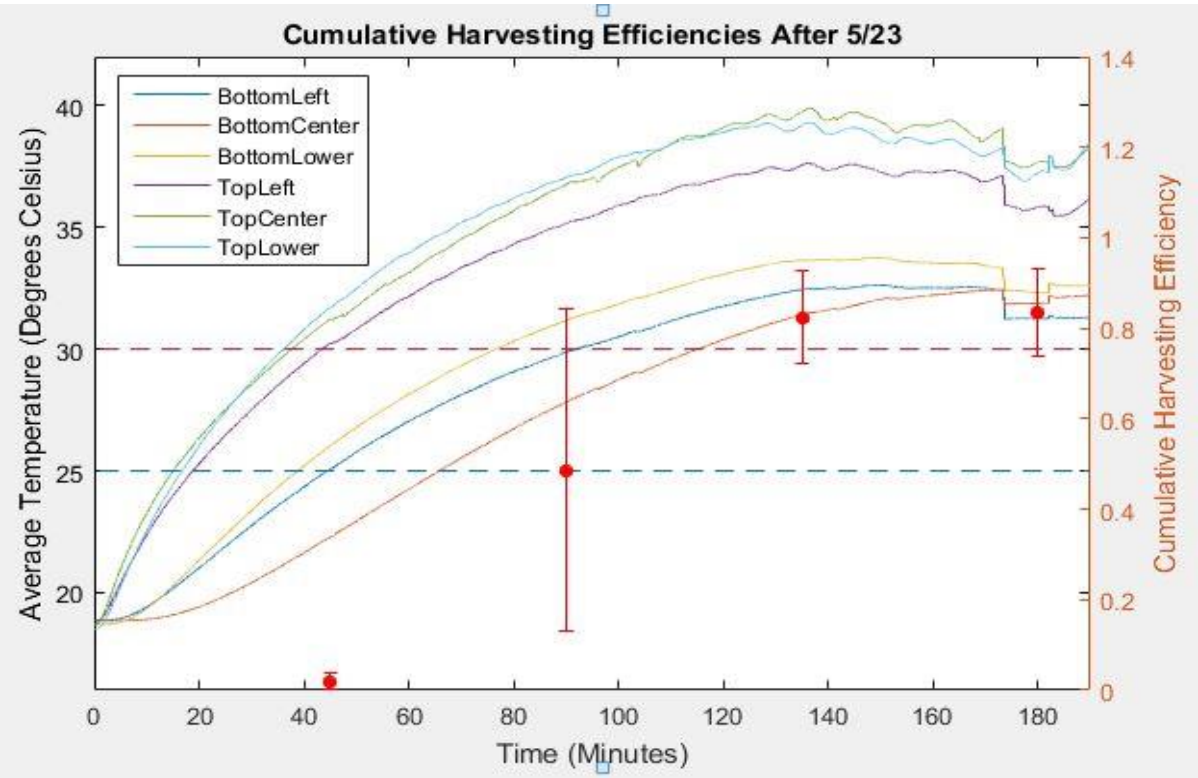
Figure 5.5. *The mean harvesting efficiency for each heating pad setting (1=low, 2=medium, 3=high) shown at 45 minute intervals along with temperature recorded every 0.5 seconds.*

Based on this first series of harvesting efficiency trials, we determined that temperature need to be controlled to maintain the soil temperatures safe for white worm eggs, as well as for automating the harvesting system. A temperature controller with a single probe, capable of being programmed to turn off at a certain temperature, was added to the prototype harvester. Two experiments were run with the probe on the bottom of the soil and set to 25 °C, and on top of the soil and set to 35 °C.

Heating the soil at the bottom of the harvester to 25 °C led to the top soil layers cooling off too quickly, which could lead to worms moving away from the heat at the bottom and migrating back to the upper layer of the soil. However, when the soil on top was heated to 35 °C, the resultant harvesting efficiency was 0.16. Therefore, in an effort to increase the harvesting

efficiency, the temperature probe setting at the soil top was increased to 40 °C. In addition, a mount for the probe was incorporated to keep it at a constant depth (5 mm) in the soil. These changes dramatically increased harvesting efficiency (Fig. 5.6).

Figure 5.6. *Mean harvesting efficiency at 45 minute intervals of six trials (red dots ± one standard deviation) of the prototype harvester set to 40 °C and with soil temperature recorded from top and bottom soil layers every 0.5 seconds*.



To evaluate the effectiveness of the modifications made to the prototype harvester, another series of trials were conducted. At least three harvesting trials were completed for each modification treatment, with the temperature controller set to 40 °C and run for 3 hours. The modification treatments consisted of: 1) both insulation and aluminum sheet [all mods], 2) insulation only [Ins], 3) aluminum sheet only [Al], and 4) neither the aluminum sheet nor insulation [no mods]. There was no statistical significance in the harvesting efficiency between any of the modification treatments set ups (one-way ANOVA, p=0.1219) but the insulation and aluminum sheet together (all mods) yielded a 16% higher mean harvesting efficiency when compared to the set up without the aluminum sheet and insulation (no mods; Fig. 5.7).

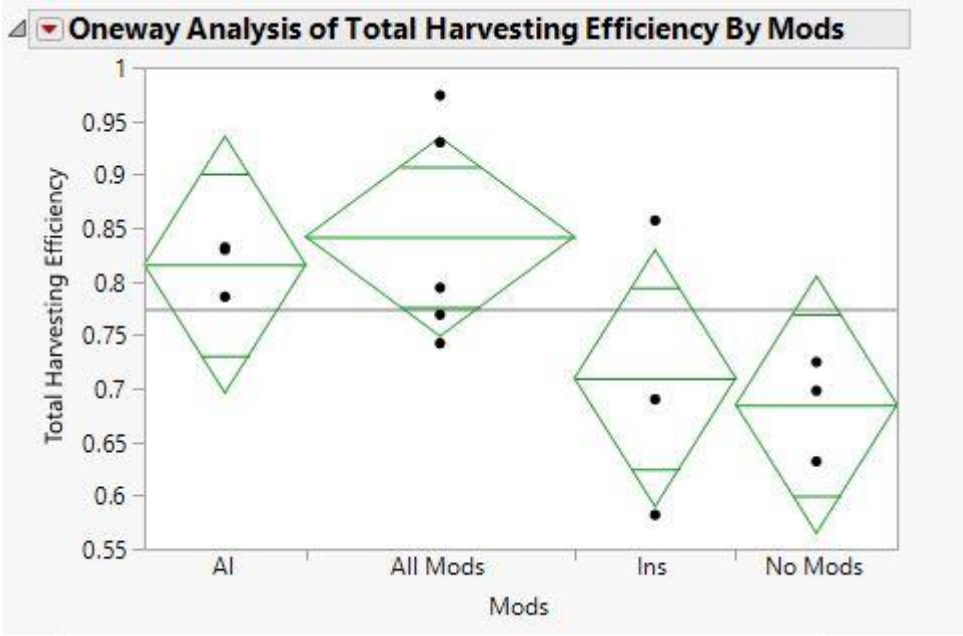


Figure 5.7. *One-way ANOVA of the mean harvesting efficiencies from each modification treatment tested. The centerline of each diamond represents the mean harvesting efficiency for each setup. The upper and lower liens in each diamond represents the standard error in the harvesting efficiency for each setup. Al = aluminum sheet only, All mods = both insulation and aluminum sheet, Ins = insulation only, No mods = neither the aluminum sheet nor insulation.*

Andrew continues to analyze the prototype worm harvesting data and make recommendations for future study. At this point, it appears of the various designs evaluated, the best overall harvesting efficiency occurs after 2.25 hrs using the prototype with aluminum and insulation modifications, 2.5 mm screen size, and surface temperature set to 40 °C.

ACCOMPLISHMENTS:

Outreach Overview

In Year 2 of the project, samples of white worms were made available to industry stakeholders to test and try in their own facilities with a variety of species. After testing the worms, the stakeholders supplied feedback by completing a survey. These surveys were analyzed to identify live white worm market(s), and from that feedback, specialized enrichment trials were conducted in Year 3 to customize the worm's nutritional profile to the predator's (namely ornamental fishes) needs. Presentations were given at Aquaculture America meetings, a paper was published in Aquaculture, and fact sheets were produced and submitted to NRAC. In addition, follow up occurred with all stakeholders who participated in the white worm research to share the project results.

Targeted Audiences

- Aquaculturists in private industry and academic institutions.
- Local food industries, especially breweries, which have waste products usable in white worm production.

Outputs:

- Based on stakeholder information from the first white worm workshop in Year 1, a list of metrics was developed for evaluating white worms as a live feed and presented as an online survey for all participants to complete after using their white worm samples.
- White worm samples were provided free during Year 2 to anyone who wanted to try feeding them to cultured or captive species. Approximately 222,530 worms were distributed to stakeholders. In addition, one starter culture (worms in media) was supplied to a grower, as well as start-up rearing instructions to several other growers.
- Contemporary methods designed to grow white worms at minimal cost were evaluated and a growing guide produced (NRAC Fact Sheet No. 223-2017).
- A prototype white worm harvester was designed, built, and tested.

Outcomes/Impacts:

Knowledge about white worms as a potential live feed amongst the aquaculture industry and scientific community has increased. A few aquaculturists have requested information about starting their own white worm cultures or have inquired to see if UNH is set up to distribute white worms.

Impacts Summary

1. **Relevance:** Issue – what was the problem?

There is a need for more diverse and nutritional live feeds. White worms show promise but not enough is known about cost-effective, scalable production techniques or nutritional profiles. In addition, the market for white worms needs to be defined.

2. **Response:** What was done?

We conducted experiments to evaluate how low- or no-cost industry byproducts affected white worm production and nutrition, and if adding enrichments would change the fatty acid profile of the worms, rendering them a more nutritious feed for cultured organisms. We evaluated if live white worms harbored any pathogens which would put aquaculture facilities at risk. We solicited feedback from stakeholders – aquaculturists in research and private domains who raise freshwater, brackish, and marine fishes – by shipping live white worm samples for them to test in their facilities. We developed live white worm shipping and receiving guidelines. We measured the conductivity properties of worm cultures (worms + media) when heated and tested other methods (besides heat) to refine and improve harvesting efficiency. We published papers, presented talks and posters, held workshops, and interacted directly with commercial ornamental growers to disseminate project results.

3. **Results:** How did your work make a difference (change in knowledge, actions, or conditions) to the target audiences?

Knowledge about white worms as a potential live feed amongst the aquaculture industry and scientific community has increased. A few aquaculturists have requested information about starting their own white worm cultures or have inquired to see if UNH is set up to distribute white worms.

4. **Recap:** One- sentence summary

White worms are an easily and cheaply cultivated, pathogen-free feed, high in protein and fat, that are readily consumed by many fishes, especially ornamentals.

PUBLICATIONS

Presentations:

Oral:

Fairchild, E. A. and J. T. Trushenski. 2018. Improving white worm *Enchytraeus albidus* nutrition for ornamental fishes. Ornamental Fish Session. The annual meeting of the World Aquaculture Society, February 19-22, 2018, Las Vegas, NV. (invited talk; accepted presentation)

Fairchild, E. A., M. Chambers, and M. L. Walsh. 2017. Do white worms have commercial potential as a feed in the ornamental industry? Ornamental Fish Session. The annual meeting of the World Aquaculture Society, February 20-22, 2017, San Antonio, TX.

Bergman, A., J. T. Trushesnki, and E. A. Fairchild. 2016. Cultivation of white worms *Enchytraeus albidus* using low- or no-cost feed resources. Aquaculture 2016. The annual meeting of the World Aquaculture Society, February 22-26, 2016, Las Vegas, NV.

Fairchild, E. A. and E. Groover. 2016. Effects of feeds and temporal cycles on white worm *Enchytraeus albidus* production. Aquaculture 2016. The annual meeting of the World Aquaculture Society, February 22-26, 2016, Las Vegas, NV.

Fairchild, E. A. 2015. Aquaculture initiatives at the Coastal Marine Lab. University of New Hampshire Department of Biological Sciences Sustainable Agriculture Seminar Series, September 18, 2015, Durham, NH.

Posters

Fairchild, E. A. and C. Giray. 2016. White worms *Enchytraeus albidus*: a pathogen-free live feed? Aquaculture 2016. The annual meeting of the World Aquaculture Society, February 22-26, 2016, Las Vegas, NV.

Peer-reviewed:

Print

Fairchild, E. A., A. M. Bergman, and J. T. Trushenski. 2017. Production and nutritional composition of white worms *Enchytraeus albidus* fed different low-cost feeds. Aquaculture 481: 16-24.

Digital

None

Non-Peer-reviewed:

Extension factsheets

Fairchild, E. A. and M. L. Walsh. 2017. How to grow white worms. NRAC Fact Sheet No. 223-2017.

Fairchild, E. A., M. L. Walsh, J. T. Trushenski, K. L. Cullen, and M. Chambers. 2017. White worms – a low cost live feed for the ornamental industry. NRAC Fact Sheet No. 224-2017.

Popular articles

World Aquaculture's editor has requested an article which we plan to write. We also worked with the science editor upon his inquiry and submitted an article for Hatchery International, however it was never published.

STUDENTS/PARTICIPANTS:

Name: Justin Roberts
Worked on project during his freshman year (Aug. – Oct. 2015).

Name: John Taylor
Worked on project during his sophomore year (Sept. 2015 – May 2016).

Name: Rachel Moore
Worked on project during her sophomore year (Sept. 2015 – May 2016).

Name: Andrew Pompeo
Whether Degree was completed during the reporting period (name, yes/no): no
New or Continuing Student: new Master's student
Capstone/Thesis Title (actual or anticipated): Design of an automated harvester to improve white worm production potential.
Date of Graduation: expected 12/2017

Name: Elizabeth Groover
Worked as summer technician May – Aug. 2016 prior to starting UFL Master's program in ornamental aquaculture.

PARTNERSHIPS

The following were unfunded partners on the project who tested a sample of live white worms in their aquaculture facilities:

- Doug Millar, TomKat Ranch, La Honda, CA
- James Candrl, Columbia Environmental Research Center, Columbia, MO
- Joe Sullivan, Tulsa, OK
- Matt DiMaggio, UFL, Ruskin, FL
- Dustin Drawdy, Oak Ridge Fish Hatchery, Plant City, FL
- Mike Bunting, Aquatic Collectors of FL, Wiamauma, FL
- Johnathon Foster, FishEye Aquaculture, Dade City, FL
- Jeff Carter, Carter's Fish Hatchery, Wiamauma, FL
- Eric Litvinoff, Marine Science Magnet High School, Groton, CT

In addition, Dr. Fiona Wilson was replaced by Dr. Tracy Keirns (UNH Survey Center) to take over the online survey tasks when Dr. Wilson assumed a new position at UNH.

When Dr. Jesse Trushenski moved from USI- Carbondale to Idaho Fish & Game, we contracted with New Jersey Feed Labs, LLC to complete white worm and feed nutritional analyses.