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THE MAINTENANCE AND CONSEQUENCES OF A LOW QUALITY DIET IN POECILIA LATIPINNA

An Undergraduate Honors Thesis submitted in partial fulfillment of the

requirements for the degree of Bachelor of Science

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WITH HONORS

by

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To: Dr. Timothy Collins, Chairperson
Department of Biological Sciences

This Undergraduate Honors Thesis in Biological Sciences, written by (Maya Jaffe), and entitled "The Maintenance and Consequences of a Low Quality Diet in *Poecilia Latipinna*," is submitted to you in partial fulfillment of the requirements for Undergraduate Honors in Biological Sciences. The Biological Sciences Undergraduate Honors Committee and the candidate's research supervisor have read this thesis. We recommend that it be approved.

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This thesis by Maya Jaffe is approved.

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ABSTRACT OF THE THESIS

THE MAINTENANCE AND CONSEQUENCES OF A LOW QUALITY DIET IN POECILIA LATIPINNA

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Professor Dr. Joel Trexler, Honors Research Supervisor

The adaptive significance of herbivory in nature is not well understood. In order to document the conditions that select for an herbivorous feeding habit, we must first understand how such a diet is maintained, and the consequences of doing so. A few studies have begun to reveal mechanisms of maintaining herbivory (i.e. selective feeding, diet mixing, etc.) and the associated life history responses (i.e. growth, reproduction, etc.) in terrestrial and marine systems; however, studies of this kind are underrepresented in the freshwater literature. In this study, I use the sailfin molly (*Poecilia latipinna*) as a model organism to examine diet selectivity and the effects of an herbivorous diet on growth. To study food selectivity, sailfin mollies were fed either disturbed or intact periphyton mats from one of three localities within the Everglades (Water Conservation Area 3B, the Gap, or Chekika). Mats are structured with palatable algal species (i.e. greens and diatoms) comprising the inner components of the mat, and unpalatable species (i.e. cyanobacteria) comprising the outer edges. Fish gut contents were analyzed for each treatment and periphyton locality. Results suggest that when provided access to the inner components of the mats, fish preferentially eat more palatable algae. In a second experiment, effects of an herbivorous diet were examined using neonate sailfin mollies. Fish were fed either commercial food flakes, commercial algae flakes, or ground periphyton, and growth rate was measured weekly, from birth to 21 days. Fish fed the commercial diets grew at a faster rate and reached a larger final size than those fed periphyton. These results suggest that a periphyton diet is limited in nutritional elements compared to a pure algae diet and herbivorous organisms feeding upon it may experience negative effects on growth. By studying the costs and benefits of herbivory in a freshwater system, this paper contributes to a larger study of the question of why herbivory would evolve as an adaptation when seemingly inefficient compared to carnivorous and omnivorous diets.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

INTRODUCTION

An herbivorous diet is thought to be inefficient when compared to carnivorous and omnivorous diets (Laspoumaderes et al. 2010). Carnivores easily incorporate nutrients obtained from their food, because their prey is of similar nutrient composition to themselves (Mattson 1980; Choat and Clements 1998). Similarly, omnivores supplement diets of basal resources with animal prey items (Behmer 2009), so that they are able to exploit times or areas of high primary production, but still obtain essential nutrients. Herbivores feed on relatively low quality food items and must compensate for the stoichiometric mismatch between food and body elemental composition (Mattson 1980; Caceres et al. 1994; Elser et al. 2000; Laspoumaderes et al. 2010). The benefits of carnivory and omnivory raise the question of why herbivores are common in nature.

Herbivory in terrestrial systems is well documented, but the focus is mainly on herbivores maintaining their diet strategy (Clements et al. 2009). A diet of mixed plants is thought to contribute to the maintenance of an herbivorous diet strategy (Simmonds et al. 1992; Bernays et al. 1994; Simpson and Rauenheimer 1996; Singer et al. 2002). Alternatively, selective feeding on higher quality food items may be important for this purpose. Aquatic herbivory studies lag behind those in terrestrial systems (Clements et al. 2009), and primarily focus on the effects of herbivory on coral reefs or the effects of plant secondary metabolites on herbivores (see Clements et al 2009). Information is missing on the composition of the herbivorous diet, and how that food is assimilated and used to either make tissues or offspring (Choat and Clements 1998; Clements et al. 2009). While previous studies in both systems have contributed to understanding the mechanisms sustaining herbivory, the costs/benefits of this diet are needed to formulate testable hypotheses of this mode of nutrition (Clements et al. 2009).

The Everglades is a unique freshwater ecosystem with low consumer diversity and abundance (Turner et al. 1999; Geddes and Trexler 2003). There are few vertebrate herbivore species even though the Everglades has abundant algal resources (Geddes and Trexler 2003; Gaiser et al. 2011; Ruehl and Trexler 2011), which suggests that herbivory is not an efficient diet strategy in this ecosystem. Periphyton mats are the prominent algal food source (Williams and Trexler 2006; Belicka et al. 2012) and are composed of green algae, diatoms, cyanobacteria, fungi, and detritus (Gaiser et al. 2004). The outer edges of periphyton mats are encrusted with unpalatable, filamentous cyanobacteria that secrete calcareous matrices (CaCO3), whereas palatable elements are located towards the interior of the mat (Donar et al. 2004; Gaiser et al. 2004). The sailfin molly is an herbivore native to the Everglades that feeds on green algae, cyanobacteria (Alkahem et al. 2007), and occasionally aquatic microinvertebrates (Harrington and Harrington 1961). Sailfin mollies are one of a few obligate vertebrate herbivores in the Everglades, primarily eating constituents of periphyton (Belicka et al. 2012). The Everglades and sailfin mollies are excellent model systems to study herbivory, because both periphyton mats and these fishes are abundant in nature and are easily studied in the laboratory. These favorable logistics allow us to experimentally examine the relationship between maintaining a low-quality diet and its consequences in sailfin mollies.

In a previous study (Geddes and Trexler 2003), omnivorous shrimp and fish were fed either intact Everglades periphyton mats (as they occur naturally), or disrupted mats where omnivores could easily access the algae with nutritional value. Results indicated that these animals were selectively feeding, as those provided to disrupted mats had gut contents with a higher volume of palatable green algae. Selective feeding may be an important mechanism for maintaining herbivory (Karasov and Martinez del Rio 2007). It may also have implications for the life history of herbivores, as the quality of food items has been shown to affect several life history metrics. For example, using *Daphnia*, Guo and Xie (2001) illustrated how a low quality diet results in tradeoffs between growth and toxin tolerance, and offspring size and number. In this study, I examined feeding selectivity and consequences of eating a low quality diet in sailfin mollies. Specifically, I examine gut contents of sailfin mollies grazing on intact and disturbed periphyton mats to determine how herbivory is maintained. Further, I test the effects of consuming periphyton (i.e. low quality diet) on growth of neonatal sailfin mollies to determine a potential consequence of such a diet. This study will contribute to the establishment of a framework from which evolutionary hypotheses of herbivory can then be developed and tested.

MATERIALS AND METHODS

Examining diet selectivity in sailfin mollies

Study system

Adult sailfin mollies were collected by dip net (mesh size 0.15cm) from a freshwater pond located in front of the Koven’s Center on the Biscayne Bay Campus of Florida International University (FIU BBC) in September 2013. This species is abundant throughout Florida in areas with fresh or brackish water (Page and Burr 1991) so this site was chosen simply based on its proximity to the laboratory. Periphyton mats were collected from three locations within the freshwater Everglades: Water Conservation area 3B (WCA 3B), the Gap (~2km area between WCA3A and 3B), and Chekika (Everglades National Park). These three localities were chosen because they have periphyton mats of varying composition and structure (Cleveland and Montgomery 2003). All experiments were conducted at FIU BBC and were approved by the Institutional Animal Care and Use Committee (IACUC Approval number: 13-029).

Experiment

Sailfin mollies were transported to the aquarium laboratory at FIU BBC in large coolers filled with aerated marsh water, where they were allowed to acclimate for 24 hours. They were then transferred to two 18.9L stock tanks where density was kept below one female fish per 3.78L. Fish were kept on a 12hr/12hr light/dark cycle and salinity was controlled at 2-3‰ by use of commercial aquarium marine salt mixture. They were fed commercial flakes (TetraMin®) for two months to standardize diet. Fish were then placed in individual 18.9L aquaria and starved for three days to standardize the level of hunger.

Intact periphyton mats (13x13 cm) were brought back from the field on ice and were allowed to acclimate to ambient temperature before being placed into the tanks. Mats were either immersed intact, with periphyton that remained as collected from the field, or were disrupted with periphyton shaken to reveal inner components. Five fish were assigned one of the two treatments per location, for a total of 10 fish for each periphyton location (N=30). Specimens were allowed to feed for 72 hr on mat material before being euthanized using MS-222 (tricainemethanesulfonate) and frozen for later gut content analysis.

Gut content analysis

Fish digestive tracts were dissected and measured to the nearest 0.1 mm using a dissecting scope. The entire digestive tract was weighed to the nearest 0.01g and standardized by body weight (0.01 g). Because sailfin mollies do not have a clearly defined stomach, the digestive tract was cut from the esophagus to the first bend of the tract (Loftus 2000). This portion of the gut was measured to the nearest 0.01 mm and contents were cleared onto a cover slip and weighed to the nearest 0.01mg. A single drop of deionized water was added and the cover slips were sealed onto slides with clear fingernail polish to obtain mounts with 1-2 week viability.

Gut contents were analyzed using a magnification of 630-1000X. Algal taxa were classified into 6 functional groups (filamentous, toxic, and coccoid cyanobacteria, filamentous and solitary green algae, and diatoms) and were quantified by calculating the number of cells per mg of gut material. Species grouped in the toxic cyanobacteria functional group have been cited as producers of harmful secondary metabolites (Wiegand and Pflugmacher 2005). Although toxic Everglades' species have not been shown to produce metabolites directly, toxins synthesized by these species (and others) have been found in Everglades periphyton mats (Bellinger and Hagerthey 2010).

Statistical analyses

Cell counts and relative abundance of the food groups within fish intestines for each treatment were compared (within localities only) using analysis of variance (ANOVA; followed by a Tukey's post hoc test) and analysis of similarity (ANOSIM), respectively. Results from ANOSIM were visualized using a non-metric multidimensional scaling (NMDS) ordination plot and cluster analysis (Figures. 4a-c). I used the Bray-curtis dissimilarity equation to compare the abundance of each group, and a distance matrix was then created where points (individuals) that are closer together in space (visualized using NMDS) are more similar to those that are further away from each other. I used ANOSIM and NMDS as described in Beals (2006). Gut content values were calculated as relative abundances, which were square root-transformed to meet the assumptions of the statistical methods we used.

Growth rate study

Study system

Gravid female sailfin mollies were collected as described above in July 2013. Fish were determined to be gravid by the presence of a black spot near the anal fin (Haynes and Cashner 1995). Fish were brought back to the laboratory and kept under the same conditions described above. They were observed every 24 hours to see if any had given birth. After parturition, newborn fish were removed from the maternal tanks. All experiments were conducted at FIU BBC and were approved by the Institutional Animal Care and Use Committee (IACUC Approval number 13-029).

Experiment

Three neonates from each brood were measured and assigned to 1 of 3 feeding treatments (5 replicates each, N =15): 1) commercial algae flakes (TetraVeggie®, min. crude protein 29.0%); 2) commercial fish meal (TetraMin®, min. crude protein 46%); or 3) ground periphyton flakes (n = 5 per treatment). Ground periphyton flakes were made from mats collected from WCA 3B. The intact mats were homogenized, spread thinly on a baking pan and placed in the drying oven at 80°F for 16 hrs. The dried crust was broken into flakes with a mortar and pestle. This process of hydration, heating, and crushing was repeated for the commercial algae and fishmeal in order to standardize the consistencies between the treatments.

Juvenile sailfin mollies were fed daily at approximately maximum ration (Table 1; Trexler 1997) and excess food and wastes removed with a fine-mesh dip net (Trexler 1997). Standard lengths were measured to the nearest 0.01mm every 7 days. At 21 days the fish were euthanized and measured using the anesthetic MS-222.

Table 1. Feeding schedule for sailfin molly neonates from birth until 21 days

AGE	Food (mg)
1	0
2	10
3	10
4	15
5	15
6	15
7	15
8	20
9	20
10	25
11	25
12	25
13	30
14	30
15	30
16	40
17	40
18	50
19	50
20	50
21	50

Statistical analysis

Absolute growth rate for each fish was calculated by subtracting the initial standard length from final standard length, and was then divided by 21 days. Similarly, growth rates for each week were calculated to determine any developmental differences in growth throughout the experiment. Differences in absolute growth rate of juveniles were examined for each food type using one way analyses of variance (ANOVA) followed by Tukey's post hoc tests. To eliminate any effects of initial newborn size across treatments, a one-way ANOVA was used to confirm individuals were the same size at the beginning of the experiment. Final size of individuals in each treatment was also compared using ANOVA.

RESULTS

Diet selectivity

The standardized intestinal weight (intestine wt/body wt) of fish between treatments (i.e. intact vs. disturbed for each locality) was not significantly different for any localities (WCA3B, p=0.196; Gap, p=0.201; Chekika, p=0.169; Figure 1). The number of cells per mg of gut material was comparable across treatments within localities (WCA3B, p=0.226; Gap, p=0.292; Chekika, p=0.270; Figure 2). Gut contents of fish fed intact and disturbed mats from WCA 3B were significantly different (ANOSIM; p=0.025; Figures 3 & 4a), suggesting that when the fish are given access to the contents inside of the mat, they feed selectively. Similarly, gut contents of fish fed Chekika mats were different between treatments (ANOSIM; p=0.009; Figures 3 & 4c). There was no statistical difference between the intact and disturbed mat treatments using mats from the Gap (ANOSIM; p=0.251, Figures 3 & 4b).

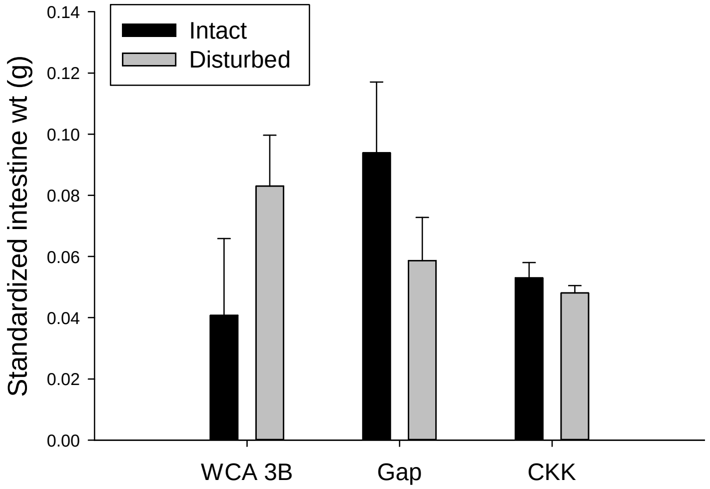


Figure 1. Standardized intestine weight (g) for both intact and disturbed treatments across localities (WCA 3B, p=0.196; the Gap p=0.201; Chekika (CKK), p=0.169. Error bars show +/-SE (p<0.05). There were no significant differences in intestinal weight of fish from all localities.

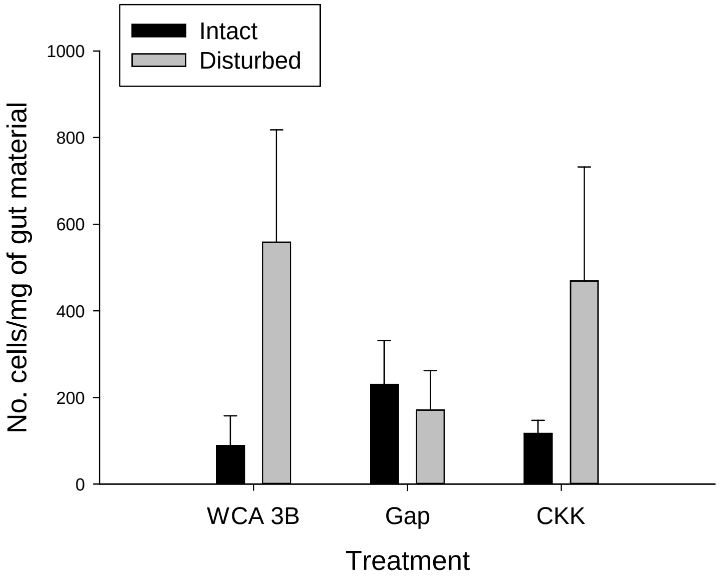


Figure 2. Number of cells/mg of gut material for both intact and disturbed treatments across localities (WCA 3B, p=0.226; the Gap, p=0.292; Chekika (CKK), p=0.270). Error bars show +/-SE (p<0.05). Gut contents within WCA and CKK were significantly different between disturbed and intact treatments, while treatments were comparable in the Gap.

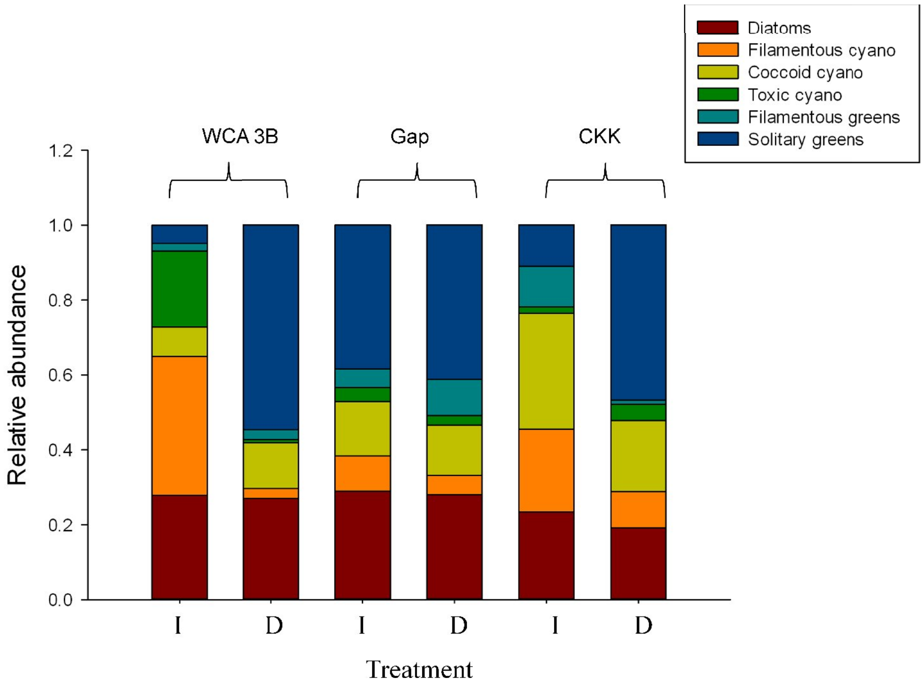
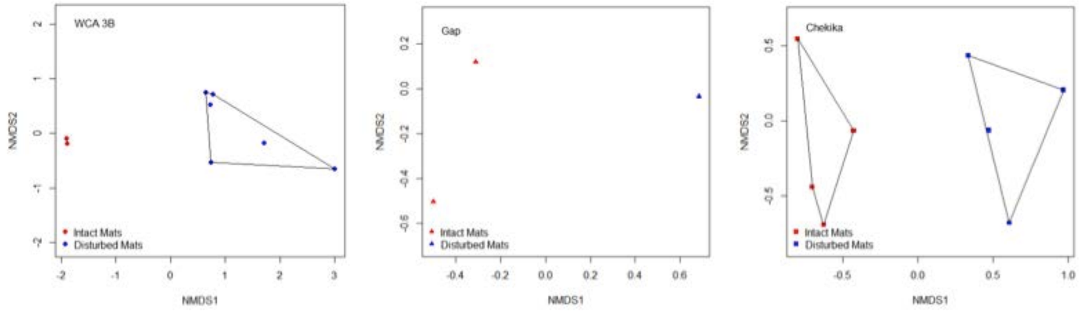


Figure 3. Relative abundance of six functional groups per treatment (I = intact; D = disturbed) across localities (WCA 3B, p=0.025; the Gap, p=0.251; CKK, p=0.009). There were significantly more green algae in the disturbed guts of both CKK and WCA treatments. Gap disturbed and intact mat treatments showed no intestinal variation.



Figures 4a-c. NMDS ordination plot (and cluster analysis) showing differences in gut composition of fish eating intact mats (red) and disturbed mats (blue) from a) WCA 3B, b) The Gap, and c) Chekika. Differences are based on relative distances of the points from each other.

Growth rate study

There was no significant difference between the initial standard lengths of neonatal fish used (ANOVA; p=0.246; Figure 5). Fish that were fed periphyton flakes had an average standard length of 10.5mm at the end of the experiment, smaller than those fed commercial algae (12.5mm) or TetraMin® diets (13.1mm) (p<0.0001).

Fish fed periphyton flakes grew at a slower absolute rate (0.078mm/day) than fed commercial algae (0.158mm/day) or TetraMin® (0.195mm/day) (ANOVA; p<0.0001). Growth rates of those fed commercial algae and TetraMin® diets were not significantly different from each other (ANOVA; p=0.203; Figure 6).

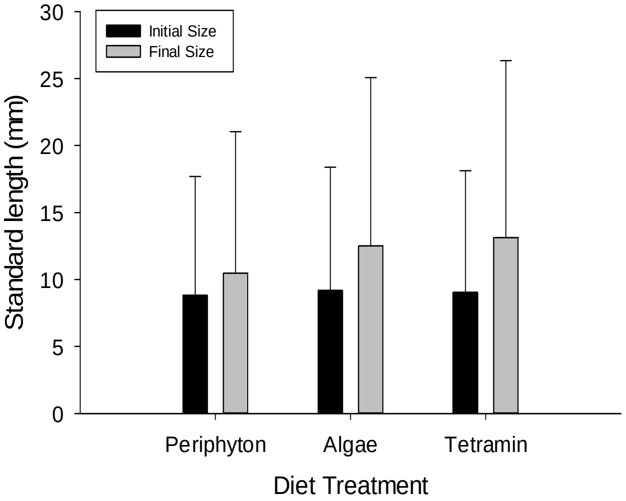


Figure 5. Initial (p=0.353) and final standard lengths (mml; p<0.001) of fish eating periphyton flakes, commercial algae, and TetraMin®. Error bars show +/-SE (p<0.05). Initial standard lengths were the same across treatments. Final standard length of periphyton treated fish was smaller than commercial algae and TetraMin®. Commercial diets were not different from each other.

When examining weekly growth, the standard length increased at a constant rate for fish in the periphyton (p=0.159) and TetraMin® treatments (p=0.882). However, for fish in the algae treatment, the growth rate in the first week (0-7 days) was different than the growth rate in the third week (14-21 days; p=0.034; Figure 7). When examining effect sizes of growth rates among treatments (differences in the mean of absolute rate between treatments), fish fed commercial algae and TetraMin ® grew 0.08 mm/day and 0.116 mm/day faster than those fed periphyton, respectively. These data provide further support that fish fed periphyton grew slower than those fed commercial diets.

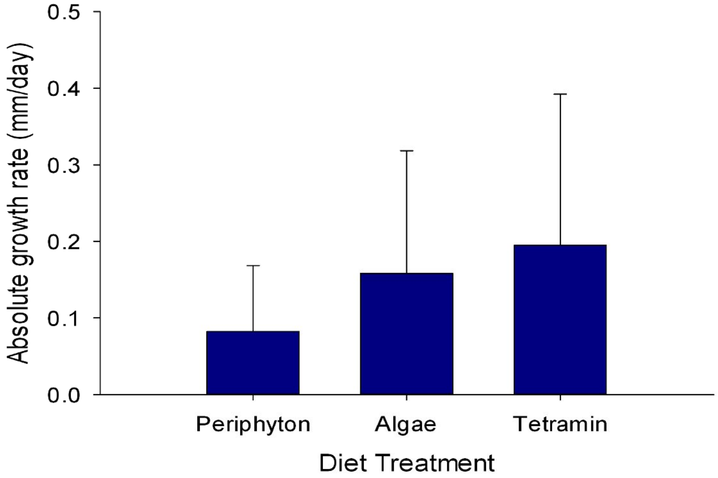


Figure 6. Absolute growth rate (final SL- initial SL/21 days) of fish eating periphyton, algae and Tetramin® (p=0.0001). Error bars show +/-SE (p<0.05). Fish fed periphyton grew at a slower rate than those fed either commercial algae or TetraMin®.

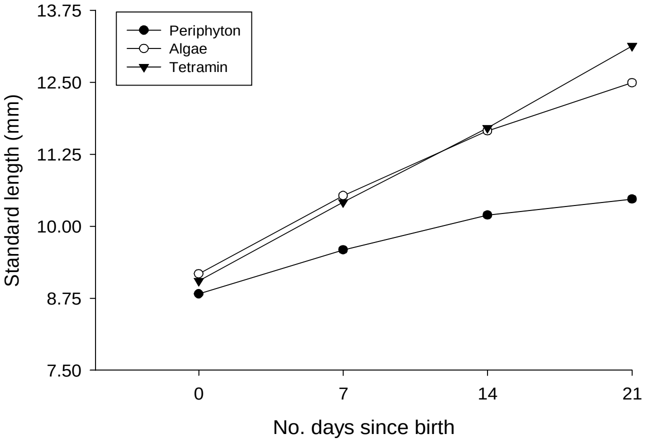


Figure 7. Weekly growth rates for fish eating periphyton, algae, and TetraMin®. Fish fed periphyton and TetraMin® displayed a constant growth rate; fish fed commercial algae showed differences in week 1 and week 3 growth (p=0.034). Effect sizes suggest that fish fed periphyton grew slower than those fed commercial diets.

DISCUSSION

By presenting a model for study of the maintenance and consequences of herbivory in aquatic ecosystems, this paper presents the first steps in studying the evolution of an inefficient diet strategy. Sailfin mollies preferentially ate green algae when available (Figure 3), suggesting that these obligate herbivores may acquire their nutritional needs through diet selectivity. By comparing the sailfin mollies' natural food source with a commercial one it was evident that their diet strategy is not ideal, which has implications for analysis of energy acquisition and allocation (Karasov and Martinez del Rio 2007). When pairing the two studies, it becomes evident that in order to maintain a seemingly inefficient diet strategy, life history trade-offs must be made that can serve as the basis for future evolutionary research.

Diet selectivity

The experiment illustrates that with a choice of food items, sailfin mollies may selectively feed on palatable food rather than eat unpalatable items on the outer edges of periphyton mats (i.e. dead algae and CaCO3-secreting filamentous cyanobacteria). This is a widely applicable feeding strategy for herbivores (Burkepile and Hay 2008), and can be suggested as a mechanism for maintaining an herbivorous diet.

While the standardized intestine weights and number of cells within each treatment did not differ among treatments, there may be important biological implications that were unseen, perhaps due to the small sample size. The fish consuming disturbed mats from WCA 3B had high concentrations of cells in their guts (Figure 2), and the intestines weighed more than fish consuming undisturbed mats from the same area (Figure 1). Because the sampled area in WCA 3B is slightly eutrophic (Gaiser et al. 2006), there may simply have been more green algae available, yielding the higher intestine weight from the sheer volume and availability of palatable food in the eutrophic ecosystem. Gut contents of fish consuming disturbed WCA 3B mats were mostly comprised of solitary green algae that are rich in protein (Karasov and Martinez del Rio 2007), when compared to diatoms and filamentous cyanobacteria seen in the fish fed intact mats (Figure 4). A higher concentration of phosphorous in the region causes dissolution of the mat structure, thus increasing the availability of green algae (Gaiser et al. 2012). Thus, the results presented here may be biased if there is in fact more green algal species in this area. If this is the case, selective feeding on algae comprising mats in this area was not observed. However, the relative abundances of algal species present in periphyton mats were not quantified, so the grazing maintenance mechanism in the Everglades remains unclear (at least in WCA 3B). Conversely, the periphyton mats collected from Chekika were thicker, with a high abundance of filamentous cyanobacteria. Green algae were preferentially consumed despite the high amount of cyanobacteria, suggesting that selective feeding may be a plausible mechanism by which herbivory is maintained in the Everglades (Figure 6).

Although not statistically significant, sailfin mollies consuming intact periphyton from the Gap had more cells/mg of gut material than those consuming disturbed mats, which is the opposite result compared to fish eating mats from the other two locations (Figure 2). Fish eating mats from the Gap also had relatively heavier guts when compared to those eating disturbed mats (Figure 1). This suggests that fish eating mats from this area are consuming more inorganic material to obtain a comparable organic, assimilable energy

(Karasov and Martinez del Rio 2007). Interestingly, fish fed mats from the Gap had digestive tracts with similar algal composition, regardless of the treatment (Figure 5). This could be attributed to the fuller guts of the fish fed undisturbed mats, compensating for the lack of readily assimilated energy sources.

Growth rate study

These data illustrate that growth rates of the sailfin mollies fed periphyton (their natural food source) was lower than both commercial treatments (Figures 6 and 7). TetraMin® (rich in energy and protein) was hypothesized to be the ideal food source, and the algae diet was chosen to represent a nutrient-poor diet relative to TetraMin® and periphyton. However, the fish had a higher growth rate when given an algal diet than the diet seen in nature. This could be because algae, while not high in energy, are rich in protein, which is important to growth in the juvenile phase. As a result, in early juvenile stages, low energy diets that are high in protein are equatable with a diet high in energy (Karasov and Martinez del Rio 2007). If this experiment were allowed to proceed until the fish reached maturation, differences in growth allocation for each treatment may have been more pronounced.

The fish fed the ideal food source (TetraMin®) grew at a constant rate, likely because this food type is not limited in any essential nutrients. Specifically, TetraMin® is formulated to provide an ample source of protein and energy (47% min. crude protein). Fish eating periphyton flakes (i.e., low-quality food source) also grew at a constant growth rate, although more slowly than those eating TetraMin®. This result may be because periphyton is low in both energy and protein; however, nutrient content of periphyton flakes is unknown, as we did not perform nutrient analysis. Interestingly, sailfin mollies fed commercial algae showed differential growth in the first and last weeks of the experiment. In nature, high quality algae have similar protein content compared to metazoan food sources, but differ in energy (Karasov and Martinez del Rio 2007).These data show that a diet high in protein, while not high in energy, may be efficient in accumulating muscle mass when growing; however, as the fish reach maturation, energy becomes more important (Karasov and Martinez del Rio 2007) and the growth rate begins to level off, as fish make tradeoffs between growth and reproduction. This pattern has also been seen in aquatic invertebrates. For example, Sterner et al (1993) found that protein limits growth of juvenile *Daphnia*, suggesting that protein is important for the growth phase of an individual’s lifecycle. However, Guo and Xie (2011) found a negative relationship between consumed protein and egg number in *Daphnia*, providing evidence that protein is likely not as important when growing ends and reproduction begins.

As evidenced by this study, selective feeding may be a mechanism for maintaining herbivory in sailfin mollies. Furthermore, consuming a low quality diet such as periphyton, has direct consequences on the life history of sailfin mollies. Since herbivory is a relatively inefficient diet strategy, understanding the maintenance and consequences of herbivory is an important first step in understanding the evolution of this challenging diet strategy in nature. This study provides a foundation to study the cost-benefit analysis of herbivory in aquatic systems, allowing for the formation of hypotheses for the evolution of herbivory as an adaptation.

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