FOOD WEB CONSEQUENCES OF A SEAGRASS MICROPARASITE AND A CRUSTACEAN MACROPARASITE

by

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DISSERTATION ABSTRACT

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Despite their ubiquity and known ecological impacts, parasites are still infrequently considered in studies of trophic ecology. Additionally, the most recognized effects of marine parasites on food webs are those caused by mass mortalities. In contrast to these density-mediated effects, trait-mediated indirect interactions (TMII), where host ecological function is altered through parasitism, are less conspicuous but not necessarily less important. In this dissertation, I present studies of potential TMIIs of two marine parasites.

The protist *Labyrinthula zosterae* D. Porter & Muehlstein (Lz) infects the eelgrass *Zostera marina* L. First, in Chapter II, I ask whether Lz may increase the nutrition of eelgrass tissue by synthesizing DHA, a nutritious ω-3 fatty acid (FA), based on Lz's relatives. By culturing Lz on various substrates, I found that Lz produces DHA as its primary FA and in detectable amounts in diseased tissue. This suggested that diseased tissue may be more nutritious for eelgrass consumers, which I tested in Chapter III using the detritivorous copepod *Tisbe* sp. Lilljeborg. Providing *Tisbe* either healthy or diseased eelgrass segments, I asked whether diseased eelgrass was functionally like detritus and fostered copepod population growth. Diseased eelgrass segments produced greater copepod numbers than healthy ones. Resulting copepods did not show clear differences

in DHA, suggesting that FA changes were less important than eelgrass material becoming more labile via disease. Nonetheless, this showed that disease may foster secondary production.

In Chapter IV, I studied the effects of the rhizocephalan *Sylon hippolytes* M. Sars infecting the shrimp *Pandalus danae* Stimpson. Using a field survey, I found that *Sylon* increased rates of epibiosis on hosts, which may interfere with shrimp antipredator defenses. Infected shrimp also showed distinct FA profiles relative to uninfected ones, with changes substantial enough to alter dietary mixing model predictions. Thus, *Sylon* may affect marine trophic interactions and our understanding of them.

Altogether, this work shows that Lz and *Sylon* can substantially alter their hosts, producing unrecognized TMIIs in their ecosystems. The results encourage further research into these systems and a greater appreciation for marine parasites in food webs.

This dissertation includes both previously published and unpublished coauthored material.

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For anyone making a better world in the way you can.

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CHAPTER I

INTRODUCTION

Parasites are an unambiguously massive fraction of biological diversity, despite difficulties in estimating parasite diversity (de Meeûs & Renaud 2002, Dobson et al. 2008, Larsen et al. 2017). Parasitism is broadly defined as a symbiotic relationship between a symbiont (the parasite) and its host, from which the symbiont extracts benefit at its host's expense (Anderson & May 1978). Parasitism is receiving increased research interest, including calls for integrating parasites into food webs (Marcogliese & Cone 1997, Lafferty et al. 2008, Jephcott et al. 2016), parasite conservation (Carlson et al. 2017), and connecting disease ecology and ecosystem science (Preston et al. 2016). Still, parasitism is still infrequently considered in studies of food webs and trophic ecology. Including parasites in food webs substantially affects their network measures, such as increasing connectance and links and influencing stability, showing parasites' integral roles in their communities (Lafferty et al. 2006, Thompson et al. 2013, Jephcott et al. 2016). The clear importance of parasites in their ecosystems, along with their ubiquity, motivate integrating parasitism into the lens of trophic ecology.

One means of bridging parasite and trophic ecology is in methodology; studies in parasite and disease ecology can benefit from the use of tools developed in food web ecology. Notably, trophic biomarkers, including chemicals, their isotopic forms, or elemental isotopes, are used to trace interactions through an ecosystem. Biomarkers can provide information about feeding relationships when they are not easily observed directly. For example, fatty acids (FA) are widely used as qualitative and quantitative signals of consumer-resource relationships (Dalsgaard et al. 2003, Iverson et al. 2004,

Iverson 2009, Kelly & Scheibling 2012, Stock et al. 2018). FA are lipid components that are unique in their structure and biosynthesis. Because certain primary producers produce mainly certain FA, researchers can trace the sources of nutrition through an ecosystem through them (Dalsgaard et al. 2003, Kelly & Scheibling 2012, Galloway & Budge 2020). Additionally, FA are themselves important nutrients; in particular, several long-chain polyunsaturated fatty acids (LCPUFA) are considered essential for many animals and must come from their dietary sources (Parrish 2009). Applying FA approaches to disease ecology can provide information about the interactions that a parasite has with its host and other ecosystem members while also revealing potential nutritional implications of the relationship.

In this dissertation, I focus on how a parasite affects trophic relationships by altering the way its host interacts with other community members. These are effects called trait mediated indirect interactions or TMIIs, in contrast to changes caused by population or numeric impacts of parasites on their hosts, known as density mediated indirect interactions or DMIIs (Werner & Peacor 2003, Abrams 2015). DMIIs are intuitive, as disease-driven decimation of a host population might essentially remove the host's influence on its ecosystem. A recent, notable example is the sea star wasting epizootic that drove the predatory sunflower star *Pycnopodia helianthoides* (Brandt) to near extinction (Hewson et al. 2014, Harvell et al. 2019). The loss of these key predators allowed urchins to flourish and in turn graze down vital kelp habitat (Schultz et al. 2016, Burt et al. 2018). TMIIs, on the other hand, are likely more subtle and less detected but not necessarily absent. Ignoring them discounts the roles parasites may have in their ecosystems.

The following chapters present three studies in two host-parasite systems. Each study investigates parasitism's effects on local ecosystems largely through the lens of trophic ecology and each using FA analysis as a tool. Chapters II and III study the first system, eelgrass wasting disease (EWD) caused by the microparasite *Labyrinthula zosterae* D. Porter & Muehlstein in its host, the eelgrass *Zostera marina* L.

Eelgrass and other seagrasses are considered foundation species because they form habitat in soft-sediment coastal ecosystems and contribute numerous ecosystem services (Nordlund et al. 2016, 2017, Lamb et al. 2017, Unsworth et al. 2019). Thus, disturbance to seagrasses can have severe consequences for other ecosystem members. In the 1930s, massive die-offs struck eelgrass beds along coasts of the northern Atlantic Ocean (Renn 1934, 1935). The declines were dramatic, such that they, though habitat loss, are credited for the first recorded extinction of a marine invertebrate, the eelgrass limpet (Carlton et al. 1991). The die-offs were determined to be caused by a parasitic protist, later described as *Labyrinthula zosterae* (hereon Lz, Renn 1936, Muehlstein et al. 1991). *Labyrinthula*, a spindle shaped cell, infiltrates eelgrass cells by moving and digesting with its ectoplasmic net, a characteristic of its broader taxon, the Labyrinthulomycetes (Muehlstein 1992, Bennett et al. 2017). Most Labyrinthulomycetes are ubiquitous in marine environments as saprotrophs (Raghukumar 2002), though others, like Lz, are known pathogens of diverse organisms including turfgrass (Bigelow et al. 2005), nudibranchs (McLean & Porter 1982), corals (Burge et al. 2012), and bivalves (Whyte et al. 1994).

The disease caused by Lz is called eelgrass wasting disease (EWD), which appears as darkened, necrotic lesions on blades (Muehlstein et al. 1991, Muehlstein

1992). EWD is commonplace and present even in apparently healthy eelgrass beds (Groner et al. 2016). In causing disease, Lz is considered an opportunistic pathogen, as it is seemingly omnipresent while only causing disease in situations where the host may become susceptible (Groner et al. 2014, 2016). Lz's ubiquity and its potential impacts to an ecologically vital foundation species incentivizes investigation of its role in local ecosystems.

To address this goal, in Chapter II, I focus the transformation of the host's FA as a potential ecological effect of Lz on eelgrass. Lz's relatives are known for producing substantial amounts of the nutritionally valuable FA docosahexaenoic acid or DHA (Sakata et al. 2000, Kumon et al. 2006, Armenta & Valentine 2013), and I hypothesized that Lz improve the FA nutrition of eelgrass for other consumers through disease. I analyzed the FA differences of diseased and healthy eelgrass tissues in the laboratory and the field to address this aim. Chapter II was published in the journal *Diseases of Aquatic Organisms* (Yoshioka et al. 2019) with coauthors Dr. Julie Schram, who assisted with FA analysis and writing, and Dr. Aaron Galloway, who assisted with writing and serves at the principal investigator.

In Chapter III, I extended my findings in Chapter II to ecosystem members beyond eelgrass and Lz. Detritus is an important form by which eelgrass enters local and nearby food webs (Valentine & Heck 1999, Valentine & Duffy 2007, Heck et al. 2008). Because Lz degrades seagrass tissue to a form that qualitatively resembles detritus (Yoshioka pers. obs., Muehlstein 1992), I used a laboratory experiment to investigate how EWD affected the availability of eelgrass biomass to the detritivorous copepod *Tisbe* Lilljeborg and the potential changes to copepod FA. Chapter III is unpublished but will

include Dr. Aaron Galloway as a coauthor, as he will assist with final manuscript preparation and serves as principal investigator.

As a consumer-resource strategy, parasitism can more specifically be defined as several modes, including parasitoids, macroparasites (typical parasites), and microparasites (pathogens), with the different modes representing similar but distinct ecological relationships with hosts (Anderson & May 1979, Lafferty & Kuris 2002). Because of their biological differences, macroparasites would not necessarily be expected to interact with their hosts and ecosystems in the same way as microparasites (Anderson & May 1979, Lafferty & Kuris 2002). Accordingly, Chapter IV investigates the relationship between the rhizocephalan parasite *Sylon hippolytes* and its host the dock shrimp *Pandalus danae*. Rhizocephalans are parasitic barnacles that, despite evolving from a filter-feeding ancestor, are morphologically reduced and specialized to infect other crustaceans (Høeg 1995, Ewers-Saucedo et al. 2019). In the Lafferty & Kuris (2002) taxonomy of parasitic life styles, rhizocephalans are more precisely termed "parasitic castrators". These parasites redirect host reproductive investments into their own growth and reproduction, often also inducing marked changes to host biology to improve parasite fitness (Høeg 1995, Lafferty & Kuris 2009).

Despite little research into its ecology, I hypothesized that *Sylon* would also significantly affect its shrimp host with potential TMIIs. As with EWD, where TMII impacts would likely scale with eelgrass's function as a foundation species, I also expected that even subtle TMIIs of *Sylon* would scale to the community level because of *P. danae*'s abundance in its ecosystems. *P. danae* is also an important prey for marine fish (Bergström 2000, Turner et al. 2017), which may also amplify *Sylon*'s effects on

other community members. Chapter IV used a field survey to examine *in-situ* differences between infected and uninfected shrimp, followed by FA analysis and a trophic biomarker mixing model exercise to infer effects beyond the parasite and host.

Chapter IV is currently unpublished but includes coauthors Suhn Brown, for their work-in-progress on behavioral changes in sylonized shrimp, Nancy Treneman, for her identification of epibiotic algae I collected from shrimp, Dr. Julie Schram, for her assistance in FA analysis, and Dr. Aaron Galloway. All coauthors will ultimately contribute to manuscript preparation.

Despite their uncommon consideration in trophic ecology, parasites can serve vital roles in affecting food web relationships. Indeed, understanding parasite roles at community and ecosystem levels remains a priority, as is contextualizing ecological patterns and processes through infection data (Marcogliese 2004, Preston et al. 2016, Gehman et al. 2019). With density-mediated effects intuitive and well-known, there is a greater motivation for uncovering less perceptible trait-mediated effects when host mortality is not a conspicuous outcome. Here, in studying key parasites of two ecologically important hosts, I investigate how parasites change host traits through infection and disease, and extrapolate their wide-reaching influence as TMIIs. Uncovering these roles underscores the necessity of considering parasites when studying food webs.

CHAPTER II

EELGRASS PATHOGEN *LABYRINTHULA ZOSTERAE* SYNTHESIZES ESSENTIAL FATTY ACIDS

From Yoshioka RM, Schram JB, & Galloway AWE (2019). Eelgrass pathogen *Labyrinthula zosterae* synthesizes essential fatty acids. Dis Aquat Organ 135:89-95. Minor editorial changes have been made for dissertation consistency.

1. INTRODUCTION

By definition, parasites and disease negatively affect their hosts. However, their effects, either direct or via modification of host traits or abundance, are multifaceted and relatively understudied at community and ecosystem scales (Preston et al. 2016). For example, parasites are recognized to modify biogeochemical processes (Breitbart 2012) and account for substantial biomass (Kuris et al. 2008). Thus, it should be a priority to understand potential effects of parasites beyond their hosts.

Eelgrass wasting disease (EWD), afflicting the eelgrass *Zostera marina* L., caused die-offs of Atlantic eelgrass beds in the 1930s (Renn 1934) and 1980s (Short et al. 1987). It is caused by the parasitic protist *Labyrinthula zosterae* Muehlstein et al. (1991, hereafter 'Lz'). EWD and related *Labyrinthula*-caused diseases are concerning with seagrasses declining worldwide (Waycott et al. 2009), especially with EWD's wide geographic breadth (Sullivan et al. 2013) and diverse seagrass hosts infected by labyrinthulids (Vergeer & den Hartog 1994). EWD can also be common within sites; In

Washington, USA, Groner et al. (2016) found prevalences >40% at over half of their sites, with a 79% EWD prevalence at one site.

Lz belongs to the Labyrinthulomycetes, heterotrophic protists characterized by their ectoplasmic net (Raghukumar 2002). The Labyrinthulomycetes include diverse pathogens (Raghukumar 2002) but are also known for abundantly producing long-chain polyunsaturated fatty acids (LCPUFA), namely eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3) (Kumon et al. 2006, Armenta & Valentine 2013). Such LCPUFA and some other fatty acids (FA), including linoleic acid (LIN, 18:2ω-6) and α-linolenic acid (ALA, 18:3ω-3), are important nutritional components for many consumers that rely on them for diverse physiological needs ranging from cell membrane fluidity to nervous system function (Parrish 2009). LIN and ALA, in particular, are considered "essential" FA, because most consumers cannot synthesize them in biologically relevant amounts (Parrish 2009). DHA and EPA are also often termed essential for the same reason, although ALA can serve as a precursor for them if the organism in consideration has elongation and desaturation abilities (Parrish 2009). Eelgrass has ALA as its dominant fatty acid but is poor in LCPUFA (Jaschinski et al. 2008, Galloway et al. 2012). While increasing evidence shows animals are more capable of synthesizing LCPUFA than recognized (Kabeya et al. 2018), increased growth and reproduction associated with greater dietary long-chain essential FA (Winder et al. 2017) indicate that exogenous sources of LCPUFA are still important. In eelgrass beds, red algae and epiphytic diatoms may supply EPA and some other LCPUFA (Jaschinski et al. 2008, Galloway et al. 2012). In contrast, DHA is uncommon in macrophytes (Galloway

et al. 2012), suggesting DHA may instead enter eelgrass beds through the water column or microbial production.

Microbial bioconversion of biomolecules has been suggested as a mechanism for "trophic upgrading" (Klein Breteler et al. 1999) in aquatic food webs. Specifically, microorganisms consume relatively nutritionally poor organisms or substrates and then synthesize more nutritionally valuable compounds for their predators. The ability for some planktonic heterotrophic protists to produce LCPUFA and other compounds is suggested to be one means of upgrading in pelagic food webs (Chu et al. 2008, 2009). Similarly, the capacity of Labyrinthulomycetes, which are typically saprotrophs, to synthesize LCPUFA is hypothesized to be a possible upgrading mechanism for detritus (Raghukumar 2002). If so, such LCPUFA production may be an important ecological role for this taxon (Raghukumar 2002).

Here we investigate an intersection of Labyrinthulomycetes' pathogenicity and FA production. We quantify the FA of Lz on three substrates in the laboratory: serum seawater agar (SSA, a typical artificial medium), an eelgrass-based medium (biologically relevant substrate), and inoculated eelgrass segments (approximation of *in-situ* production). We use these substrates to determine how *in-vitro* FA production of Lz might vary with different resources and whether Lz FA production might be detectable in diseased host tissue. To assess whether substantial FA production may occur *in situ*, we also quantify FA of field-collected EWD-affected eelgrass. We focus particularly on ALA and DHA, hypothesizing that Lz presence will increase sample DHA at the expense of ALA, a key eelgrass FA and potential precursor for DHA.

2. MATERIALS AND METHODS

We collected EWD lesions for Lz isolation and eelgrass for experimental substrates in the South Slough in Charleston, Oregon, USA in 2017 and 2019. We collected only eelgrass leaves (i.e. not whole turions) under State of Oregon Parks and Recreation Department permit #008-16 and with permission of the South Slough National Estuarine Research Reserve. We performed laboratory work and FA analyses at the University of Oregon Institute of Marine Biology, Charleston, Oregon, USA.

2.1. Substrate and sample production

We prepared three types of substrates in 100-mm petri dishes: SSA, eelgrass agar (EGA), and eelgrass leaf segments (hereafter, "segments"), summarized in table 2.1 and detailed in Supplement 1 of Yoshioka et al. (2019). We used nine isolates (V17-V25) of Lz, cultured from EWD lesions. We aimed to capture a greater variety of Lz FA production using multiple isolates rather than a single isolate but did not sample to specifically test isolate differences. We inoculated four replicate plates of each substrate with 10 uL of ~10^6 cells mL^{-1} of each Lz isolate in sterile seawater. Four replicate control plates for each substrate were treated the same but using sterile seawater as a sham inoculum. EGA did not initially yield sufficient Lz growth, so we plated pieces of Lz-colonized EGA onto new EGA plates. Isolate V22 still did not grow sufficiently and was omitted. We incubated all plates at 15.6 ± 0.34°C (mean ± sd) with 12/12-hr light/dark fluorescent lighting. We allowed dark lesions typical of EWD to develop on inoculated segments before collection. Incubation durations varied with extremely different timings

of disease/degradation on segments and substantial Lz growth on agar substrates (10 days for SSA, 42 days EGA, and 8-31 days segments).

Table 2.1. Summary of Lz substrates and sample types.

Substrate	Sample types	Eelgrass ?	Other components	Sterilization	Relevance
Serum seawater agar (SSA)	Lz cells, Lz-colonized substrate, substrate alone	None	Horse serum, peptones, yeast extract, glucose, germanium dioxide, noble agar	Heating (dissolve agar), autoclaving	Traditional artificial medium used to culture Lz
Eelgrass agar (EGA)	Lz-colonized substrate, substrate alone	2nd/3rd leaves of turion, cleaned of epibionts and blended	Filtered seawater, noble agar	Heating (dissolve agar), autoclaving	Medium plate with only eelgrass-derived resources without eelgrass host response
Eelgrass	Laboratory-inoculated segments: Lz-colonized substrate, substrate alone	7-cm piece of eelgrass from 2nd/3rd leaves of turion, cleaned of epibionts	Sterile seawater, sterile seawater agar plates	None	Controlled but realistic production of EWD-lesioned tissue
	Field-collected tissue: field diseased, field healthy	Leaves cleaned of epibionts, portions of diseased and healthy tissue separated	None	None	in-situ FA production

From SSA plates, we collected Lz-colonized agar from one inoculated plate per isolate (Lz-colonized substrate, n = 8) and agar from all control plates (substrate alone, n = 4). We also scraped cells from all inoculated SSA plates and pooled them by isolate (Lz cells, n = 8) to quantify FA per Lz weight and separate Lz FA from that in associated agar. From EGA plates, we collected paired colonized (Lz-colonized substrate, n = 8) and uncolonized (substrate alone, n = 8) agar. EGA samples used paired controls because another set of four separate control plates could not be produced due to the additional plating as described and limited quantities of EGA plates. Lz growth on EGA was compact enough to allow collection of distinct colonized and uncolonized samples from each plate.

Before collecting inoculated (Lz-colonized substrate) and control (substrate alone) segments, we photographed and quantified each segment's EWD severity as the percent segment area lesioned using ImageJ (Rasband 1997). We stored segments separately but pooled them for FA analyses to ensure necessary sample masses. Because pooling reduced control sample size to n = 1, we later produced a set of inoculated and control segments (n = 5 each) using larger paired segments (each pair from the same leaf) and only the high-virulence isolate V20. Methods were otherwise the same. We stored and analyzed each of the later segments individually (i.e. not pooled).

To assess whether FA of inoculated segments reflect those of EWD lesions *in situ*, we additionally collected five eelgrass leaves with eelgrass lesions. We excised apparently diseased (lesioned) tissue from healthy tissue, storing each separately (field diseased and field healthy, resp., n = 5 each). We verified visual EWD diagnosis by plating small subsamples of diseased tissue on SSA.

We freeze-dried and weighed all samples, obtaining dry sample weights, prior to FA extraction. We extracted and analyzed FA using gas chromatography and mass spectrometry, modifying methods of Taipale et al. (2013, 2016) as detailed in Supplement 1 of Yoshioka et al. (2019).

2.2. Data analysis

We analyzed data in R (version: 3.5.1). We standardized FA concentrations by sample dry weight (μg mg^{-1} DW) and calculated FA proportions on the total identified-FA concentrations within a sample (%TFA). We used nonparametric methods for univariate comparisons, as data were non-normal with small sample sizes. We compared

DHA and ALA concentrations and proportions among sample types within substrates. We used Wilcoxon Signed Rank ('Wilcoxon' hereafter) tests for EGA and eelgrass, as samples were paired, and Kruskal-Wallis ('KW' hereafter) tests for SSA. To address the specific hypothesis that Lz would increase DHA at the expense of ALA, we used one-tailed Wilcoxon tests for eelgrass samples but also present two-tailed tests for transparency. We did not adjust these tests for multiple comparisons because each deliberately investigated a particular FA's patterns within a substrate. We used Dunn's tests with Bonferroni corrections for posthoc pairwise comparisons following KW tests (dunn.test in package dunn.test, Dinno 2017).

We used Euclidean distances of arcine-square-root-transformed proportions for multivariate analyses, including FA >1% on average of FA of any substrate-sample type combination (23 FA used). We visualized FA multivariate compositions using non-metric multidimensional scaling (NMDS, metaMDS in vegan package, Oksanen et al. 2018) and compared them among substrates and sample types using permutational multivariate analysis of variance (PERMANOVA with 9999 permutations, adonis in vegan package, Oksanen et al. 2018).

We do not display or analyze the earlier segment data as the control had n = 1 due to pooling. Further, temporal and methodological differences between earlier and later segment sets discourage combining them, although they are qualitatively similar (Figure S1 in Supplement 1 of Yoshioka et al. (2019)).

3. RESULTS

All field-collected EWD samples produced Lz growth on SSA plates.

DHA was the dominant FA in SSA- and EGA-cultured Lz samples, followed by palmitic acid (16:0). In SSA-cultured Lz cells, DHA composed 36.6 ± 11.6% (mean ± sd, n = 8) of identified FA. Palmitic acid was the dominant FA in SSA and EGA substrate-alone samples. ALA was the dominant FA of all eelgrass samples.

On SSA, Lz cells had proportionally greater ALA than the substrate alone (Dunn's test, $p < 0.05$, Fig. 2.1A). In contrast, ALA concentrations were greater in Lz cells than both Lz-colonized SSA or SSA alone (Dunn's test, $p < 0.05$, Fig. 2.1C). For EGA, Lz-colonized substrate had greater ALA proportions and concentrations than the respective controls (Wilcoxon, $p < 0.05$, Fig. 2.1A, C). In both laboratory-inoculated and field-collected eelgrass, ALA proportions and concentrations were lower in Lz-inoculated and diseased ones relative to respective controls (one-tailed Wilcoxon, $p < 0.05$; two-tailed $p = 0.0625$, Fig. 2.1A, C).

DHA proportions were not different between Lz cells or Lz-colonized substrate on SSA (Dunn's test, $p=1.0$), but both were greater than the SSA alone (Dunn's test, $p<0.05$, Fig. 2.1B). DHA concentrations were greater in Lz cells than both Lz-colonized SSA and SSA alone (Dunn's test, $p<0.05$, Fig. 2.1D). As with ALA, DHA proportions and concentrations were greater Lz-colonized EGA than EGA alone (Dunn's test, $p<0.05$, Fig. 2.1B,D). Eelgrass DHA showed an opposite pattern to ALA; Lz-inoculated segments and diseased tissue both had greater DHA proportions and concentrations than respective controls (one-tailed Wilcoxon, $p<0.05$; two-tailed $p=0.0625$, Fig. 2.1B, D).

We summarize univariate comparisons of proportions and concentrations in Tables S1 and S2, respectively, in Supplement 2 of Yoshioka et al. (2019).

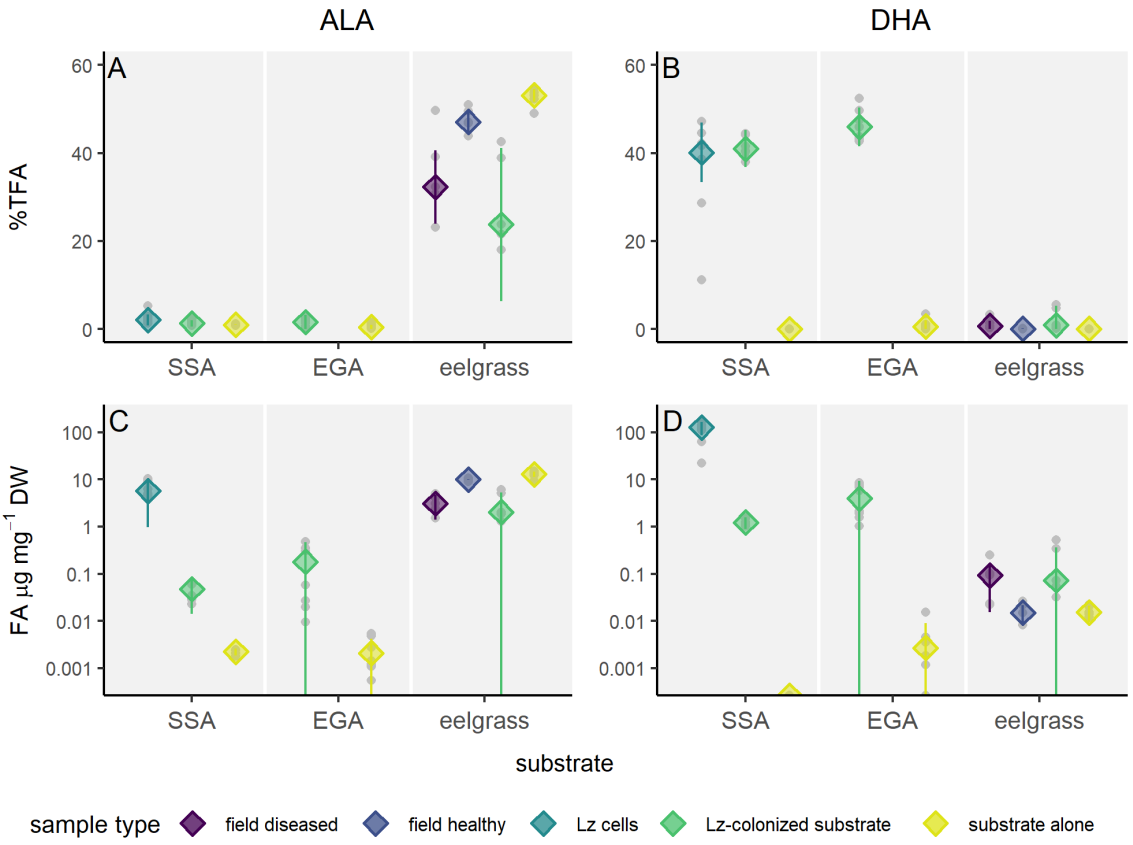

Fig. 2.1. (A, C) ALA (α-linolenic acid, 18:3ω-3) and (B, D) DHA (docosahexaenoic acid, 22:6ω-3) by substrate and sample type. Median (A, B) proportions (% total identified fatty acids) and (C, D) concentrations (µg mg^{-1} sample dry weight) are shown as diamonds with error bars (± 1 interquartile range). Substrates: serum seawater agar (SSA), eelgrass agar (EGA), and (eelgrass) leaf segments or tissue. Sample types: field-collected diseased eelgrass, field-collected healthy eelgrass, isolated *Labyrinthula zosterae* (Lz) cells, Lz-colonized substrate, and substrate alone. Grey points are individual analyzed samples. Sample sizes for each substrate-sample type combination are n = 5 for each eelgrass-sample type combination, n = 4 for SSA-substrate alone, and n = 8 for all other combinations. Data analyses are summarized in Tables S1 and S2 in Supplement 2 of Yoshioka et al. (2019).

The FA compositions of samples differed by substrate and sample type (PERMANOVA, substrate: $F(2) = 159$, partial $R^2 = 0.327$; sample type: $F(4) = 125$, partial $R^2 = 0.513$, Fig. 2.2).

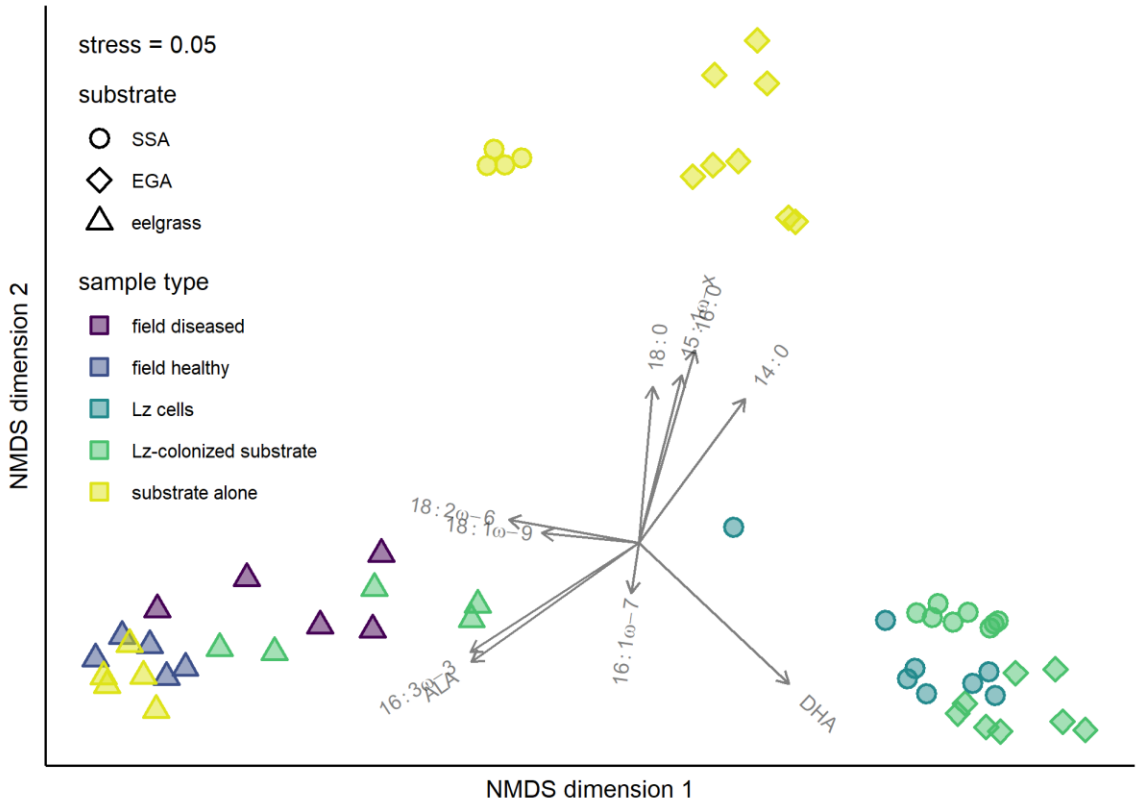

Fig. 2.2. Non-metric multidimensional scaling (NMDS; on Euclidean distances of arcsine-transformed FA proportions) plot of fatty acid (FA) compositions by substrate and sample type as in Fig. 1. Each point is an individual analyzed sample. Vectors show relative correlations of FA (>5% total identified FA of at least substrate-sample type combination) with NMDS dimensions. In vector labels, "x" denotes an unknown ω double bond position for the FA.

4. DISCUSSION

Using both artificial (SSA) and eelgrass-derived (EGA) substrates, we show that pathogenic Lz produces DHA as its dominant FA. Notably, as the agar substrates provided here lack significant quantities of even precursor FA, our results indicate Lz is capable of synthesizing and elongating many FA to produce DHA. Furthermore, while substrate was a significant predictor of FA composition in PERMANOVA, sample type explained much more of the variation (partial R^2 = 0.327 versus 0.513, respectively) and

suggests FA production of Labyrinthulids on artificial substrates may qualitatively reflect the abilities of those organisms *in situ*.

Lz's DHA content far exceeds that of most primary producers. In an analysis of 40 northeast Pacific marine macrophyte species spanning 21 orders in 4 phyla, no species exceeded on average 5 %TFA of DHA and only two had detectable DHA (Galloway et al. 2012). Field diseased eelgrass samples found here had 1.19 ± 1.26 %TFA (mean ± sd, n = 5), placing it among the top macrophyte DHA sources in the northeast Pacific. For marine phytoplankton, Galloway & Winder (2015) synthesized data for FA contents in 208 species and found the greatest DHA content in dinoflagellates (21 ± 1 μg mg^{-1} DW, 17 ± 9 %TFA, mean ± sd), exceeded by Lz cells on SSA in our study (111.9 ± 46.8 μg mg^{-1} DW, 36.6 ± 11.6 %TFA, mean ± sd). Lz's DHA content does, expectedly, fall with many other heterotrophic protists researched for industrial LCPUFA production, including Lz's relatives (5 to over 50% lipid, Kumon et al. 2006, Armenta & Valentine 2013). With greater ecological relevance, five planktonic heterotrophic protists studied by Chu et al. (2008, 2009) fed biologically relevant diets (bacteria or phytoplankton) contained DHA at ~5-17 %TFA.

In eelgrass, patterns of laboratory segments and field-collected tissue were similar (Figs. 2.1, 2.2). The culturing of Lz from field-collected lesioned eelgrass confirms our identification of EWD in the field, supporting the agreement between laboratory and field samples. The eelgrass results imply a shift in ALA and DHA with infection, with ALA being reduced and DHA being produced. A straightforward explanation could be that Lz converts ALA to DHA, but because we did not specifically trace ALA's fate, we cannot definitively conclude this mechanism. Also, we emphasize that the loss of ALA and gain

of DHA is not one-for-one, as ALA is likely being converted to other FA or being lost in degradation accompanying disease. Disease-associated degradation also likely explains the difference in ALA patterns between agar substrates and eelgrass: while ALA could be increased in ALA-poor agar substrates with Lz presence, the ALA could be lost in ALA-rich eelgrass though degradation.

While Lz-associated DHA increases found in both laboratory and field eelgrass were detectable, they were modest (Fig. 2.1B, D), which may indicate minimal relevance *in situ*. However, considering eelgrass's abundance as the foundation species in its ecosystems, the ubiquity of EWD (Sullivan et al. 2013), and the general scarcity of DHA in eelgrass bed primary producers (Kharlamenko et al. 2001, Galloway et al. 2012), even this small production may be significant for eelgrass beds. Overall, our results highlight a potential role for Lz in secondary production in its communities, but ecosystem-level effects must still be confirmed. If abundant enough, Lz could be an important upgrader (Klein Breteler et al. 1999) of LCPUFA-poor eelgrass.

Beyond considering FA production at community or ecosystem scales, investigating the potential relationship between disease severity and FA production should be a priority for future study. Specifically, investigations into the utility of FA production for pathogenic Lz may provide clues to how strain-specific virulence is modulated. Thraustochytrids, relatives of Lz, use FA like DHA for storage and the ectoplasmic net (Jain et al. 2007), an important component of infection for Lz (Muehlstein 1992). Thus, FA production may be linked to disease in mechanism, not simply pathogen load.

We here demonstrated that a marine parasite produces exceptionally large amounts of DHA, finding that Lz may be an underrecognized source of LCPUFA in eelgrass ecosystems. While unsurprising in light of its relatives (Kumon et al. 2006, Armenta & Valentine 2013), Lz's FA production is remarkable in expanding parasitism's potential ecological effects. If Lz's FA production scales *in situ,* this parasite may trophically upgrade food quality to consumers of its host via disease. This encourages studies on FA production in relation to disease in living plants to elucidate FA consequences of EWD. Future research on the trophic pathways of diseased tissue are needed to show whether Lz FA production enters higher consumers via enhanced eelgrass nutritional quality. This work demonstrates that EWD may not just be a destructive force: through producing valuable biomolecules that can regulate organismal and ecosystem production (Winder et al. 2017), Lz could be a creator as well.

BRIDGE

In Chapter 2, I showed that Lz produces DHA as its primary FA, revealing the possibility that this pathogen could serve as a nutritive source while also acting as a disease agent. However, this study was limited to only the pathogen and its host, excluding other community members that may be affected by the nutritive changes. In the next chapter, I built upon Chapter 2's results, introducing the detritivorous copepod *Tisbe* and asking whether EWD could produce nutritive resources for consumers in eelgrass beds and whether *Tisbe* could directly consume Lz.

CHAPTER III

WASTING DISEASE MOBILIZES EELGRASS BIOMASS FOR DETRITIVOROUS COPEPODS

This work will include coauthor Dr. Aaron Galloway as principal investigator and contributor to the final manuscript preparation. Otherwise, I am the sole contributor of this work to date.

1. INTRODUCTION

Parasites and pathogens are well known to affect the trophic dynamics of their ecosystems by altering the biology of their hosts. Horsehair worms shunt terrestrial nutrition to aquatic systems by manipulating their cricket hosts to jump into rivers (Sato et al. 2012). Trematode cercariae link benthic lipids to planktonic consumers as they leave their snail hosts and are consumed by other aquatic organisms (McKee et al. 2019). Viruses infect and kill massive proportions of marine microbes, releasing their nutrients and driving marine biochemical cycling (Fuhrman 1999, Wilhelm & Suttle 1999). Despite examples like these, parasites and pathogens are often singly recognized for causing disease.

Eelgrass wasting disease (EWD) afflicts the eelgrass *Zostera marina* L. EWD is caused by the protist *Labyrinthula zosterae* (D. Porter & Muehlst. in Muehlstein et al. 1991, hereafter Lz). The disease manifests as dark, necrotic lesions on eelgrass leaves. In the 1930s, EWD caused massive die-offs of eelgrass along North American and European coasts and later resurfaced in die-offs in the 1980s (Renn 1934, 1935, Short et

al. 1987). EWD is common and widespread, which is concerning as eelgrass serves as a valuable foundation species on temperate coastlines and as seagrasses decline worldwide (Waycott et al. 2009, Sullivan et al. 2013).

As biogenic habitat, eelgrass provides structure in the water column that would otherwise be absent in soft sediment habitats (Heck & Orth 1980, Moore & Short 2006). Additionally, eelgrass is consumed by grazers, though this trophic contribution to modern seagrass ecosystems may not be as great as in the past (Heck & Valentine 2006, Valentine & Duffy 2007). Still, substantial seagrass biomass can enter both local and nearby food webs as detritus (Heck et al. 2008). The separated and decomposing seagrass tissue can be consumed by detritivores such as microbes, worms, and crustaceans (Blum et al. 1988, Heck et al. 2008, Reynolds et al. 2018). These organisms can then be fed upon by higher consumers. Harpacticoid copepods, for example, link detrital eelgrass biomass to juvenile salmon (Sibert et al. 1977). In some cases, harpacticoid copepods, including the genera *Harpacticus* Milne Edwards H., *Zaus* Goodsir, and *Tisbe* Lilljeborg, made up over 80 to 90 % of food items found in juvenile chum salmon, *Oncorhynchus keta* (Walbaum) (D'Amours 1987, Simenstad & Cordell 2000, Healey 2011, Kennedy et al. 2018).

We hypothesized that EWD makes live eelgrass tissue functionally like detritus by causing death to the tissue and enzymatically degrading it (Muehlstein 1992). If this is the case, diseased eelgrass tissue should support the growth and reproduction of detritivores. We first verified whether the harpacticoid copepod *Tisbe* could consume Lz, as *Tisbe* benefit from EWD could be either or both through feeding on detritus (eelgrass biomass) or Lz cells alone. We then compared the population growth of *Tisbe* copepods

provided either healthy or diseased eelgrass tissue. Specifically, we predicted that diseased eelgrass tissue would support a faster population growth and a larger end population size. Because Lz produces the nutritionally valuable ω-3 fatty acid DHA as its primary fatty acid (FA, Yoshioka et al. 2019), we lastly compared the FA profiles of the copepods raised on either eelgrass tissues.

2. MATERIALS AND METHODS

All work was conducted under institutional COVID-19 safety approval and guidelines for safe research practice.

2.1. Experimental organisms and infrastructure

We used the *Labyrinthula* culture V20 for this experiment, which was used previously (Yoshioka et al. 2019) to readily produce EWD lesions on eelgrass leaves and was isolated in 2017 from an EWD disease lesion at Valino Island (South Slough Estuarine Research Reserve, Charleston, OR, USA). We maintained this culture using a modified serum seawater agar medium (SSA) as described in Yoshioka et al. (2019). All laboratory work was performed at the Oregon Institute of Marine Biology (OIMB, University of Oregon, Charleston, Oregon, USA).

We collected various copepods and other small epifauna from the eelgrass bed in the Portside Mudflats (also known as the "Charleston Triangle", 43.3435°N, 124.3230°W, Charleston, Oregon, USA) by washing eelgrass over a 250-µm sieve, under Oregon Department of Fish and Wildlife permit #22599. We sorted and removed gravid *Tisbe* sp. from the caught organisms under a dissecting microscope and placed them into

nine half-pint glass canning jars filled with 200 mL of 0.2-μm-filtered seawater (FSW). Four animals were placed into each jar, which was racked in a flowing sea water table at OIMB to maintain temperature. Jars were initially kept separated to detect and cull non-*Tisbe* animals. Eventually, the copepods were consolidated into a single, 6-L culture housed in a modified beverage dispenser gently aerated at approximately 2 bubbles per second. This culture was maintained and subsequent work performed in a temperature-controlled room set to 15˚C (actual temperature: 15.2 ± 0.7 ˚C, mean ± sd).

We established *Rhodomonas lens* Pascher & Ruttner and *Dunaliella tertiolecta* Butcher microalgal cultures from those of G. von Dassow and R. Emlet at OIMB. We maintained the cultures with autoclave-sterilized commercial f/2 medium (Fritz Aquatics, Mesquite, Texas, USA) in batch culture until we established semi-continuous culture. In semi-continuous culture, we seeded three, 1-L Erlenmeyer flasks filled with 900 mL of f/2 medium with each *Rhodomonas* or *Dunaliella*. The flasks were gently aerated and were kept under fluorescent lighting (12:12 hr light:dark cycle). Every three or four days, we removed 300 mL from each flask and replaced it with sterile, fresh f/2 medium and fed *Tisbe* the algae with a 1/3rd water change with FSW.

2.2. *Tisbe* consumption of Lz

To validate that *Tisbe* could consume Lz, we collected *Tisbe* from the culture vessel, moved them through FSW twice to remove hitchhiking biota, and introduced them to slide with a clay-footed coverslip (about 5-10 *Tisbe* slide^{-1}), which allowed *Tisbe* to move freely and interact with Lz cells. Lz cells were harvested from colonized SSA plates and then introduced to the *Tisbe* on slides. We observed the *Tisbe* with *Lz* under a

Leitz Laborlux D microscope (10x magnification) and recorded confirmed feedings on video with a mounted Nikon D7100 DSLR camera (1920x1080, 30p).

2.3. Eelgrass wasting disease and *Tisbe* population growth

We collected eelgrass leaves from Portside Mudflats, taking one leaf, either the 2nd or 3rd, per plant (n = 10 leaves, no whole plants) under State of Oregon Parks and Recreation Department permit #008-16, which governs local marine plant and algal take. Leaves were absent of any EWD lesions or other visible damage, except for minute lesions on the tips of two leaves; the tips were discarded. We cleaned collected eelgrass leaves of epibionts by gently wiping them with lab tissue and rinsed with sterile seawater (SSW, autoclaved FSW). Two 8-cm sections of the leaves (hereon "segments") were cut from the middle of each leaf, thus avoiding the oldest and youngest parts of the leaves. We randomly assigned one of the leaf segments to the diseased treatment and the other to the healthy (control) such that an even number of distal (older) or proximal (younger) segments were in each treatment. We placed the segments individually into 50-mL centrifuge tubes filled with 45 mL of FSW. We placed the randomly-racked tubes under a daylight-temperature fluorescent lamp and allowed 24 hours for the segments to acclimate. A transparent acrylic sheet was placed over the tubes to limit evaporation. We prepared an inoculum of Lz harvested from colonized SSA plates by suspending the cells in SSW. We enumerated the cells in the inoculum and dosed the segments/tubes assigned to the diseased treatment with 1.0×10^6 cells. As a sham inoculum, we dosed the healthy tubes with the equivalent volume of the same SSW. We allowed the segments to be infected overnight.

We randomly assigned sets of 10 gravid *Tisbe*, rinsed of hitchhiking biota as previously described, to each of twenty, one-quart jars, filling each jar with 500 mL of FSW. One eelgrass segment was added to each jar (discarding any inoculation medium), producing paired, replicate jars of diseased and healthy treatments ($N_{pairs} = 10$). We lidded each jar with a clean 100-mm plastic petri dish cover to limit evaporation. Covers were vented to allow gas exchange. The jars were kept in the same temperature-controlled room under the same fluorescent lamp over the course of the experiment. On day 14, we removed segments, removed and replaced 50% of the water by reverse filtration (48 μm), and added new healthy or infected segments (depending on treatment and prepared as before). We did not change the water more frequently as this would have removed dissolved or fine materials released from segments that could contribute to resources available to the *Tisbe*. We noted the appearances of the first three gravid *Tisbe* in each jar, and collected them to assess brood sizes by counts of hatched nauplii. However, our vessel (a 48-well plate) prevented accurate counts and the method was abandoned (no data). Thus, each jar had three gravid *Tisbe* removed. We did not otherwise quantify the *Tisbe* in the jars until the experiment's end to avoid disturbance from frequent sampling. Jars were monitored every three or four days, which was the greatest frequency allowed in our COVID-19 research safety plan, which limited personnel access to OIMB facilities during this time.

After 28 days, we removed 100 mL of the water from each jar by reverse filtration (48 μm) and added 100 mL of carbonated SSW to narcotize the copepods in the jar (Gannon & Gannon 1975). The contents of each jar was then divided into two subsamples using a Folsom plankton splitter. We concentrated one subsample of each jar

over a 48-μm sieve for population composition and counts and the other over a 150-μm sieve for fatty acid (FA) analysis. We used only larger copepodites and adults for FA analysis as it was impractical to separate nauplii from feces by sieving. The population subsamples were preserved in 4% formalin in seawater buffered with sodium borate and the FA subsamples were frozen at -80 ˚C, both in glass scintillation vials.

We counted the number of *Tisbe* nauplii, copepodites, non-gravid adults, and gravid adults for each population subsample using a Ward plankton wheel under a dissecting microscope. We considered copepods adults if their length, excluding caudal setae, was 750 μm or longer, based on the lengths of gravid copepods.

2.4. Fatty acid analysis

After overnight at -80 ˚C, we freeze-dried the FA subsamples for 48 hours, added 1 mL of chloroform, and capped each vial under N_2 gas. We stored them at -20 ˚C until FA extraction, at which point we added 1 mL of methanol, 0.75 mL of 0.9% NaCl in water, and an internal C19:0 standard. The contents of each subsample were vortexed and then transferred to glass centrifuge tubes. We rinsed each vial with 1 mL of chloroform, and transferred the rinse chloroform to its respective centrifuge tube. We then carried out lipid extraction and transesterification following the methods of Taipale et al. (2013, 2016) modified to account for small sample mass. Briefly, the contents of each centrifuge tube were sonicated in an ice bath, vortexed and centrifuged for extraction. The chloroform layer containing lipids was transferred to a new vessel, and 1 mL of chloroform was added and the extraction process was repeated. We combined the two chloroform layers as lipid extract and transesterified the entire extract by evaporating to

dryness under N_2 gas and adding toluene and 1% sulfuric acid in methanol. The samples were heated at 90 ˚C for 90 minutes, after which the sulfuric acid was neutralized with the addition of $KHCO_3$, and 2 mL of hexanes were added. We vortexed and centrifuged the samples, removing the hexanes layer containing fatty acid methyl esters (FAMEs), and repeating again with another 2 mL of hexanes added. The hexanes layers containing the FAMEs were combined, evaporated to dryness under N_2 gas, and resuspended in 1 mL of hexanes. We transferred the FAMEs to autosampler vials, capped under N_2 gas, and analyzed the samples using a Shimadzu QP2020 gas chromatographer-mass spectrometer (GC-MS). GC-MS operation and FA identification were as in Yoshioka et al. (2019) and Taipale et al. (2013, 2016).

2.5. Data analysis

All analyses were performed in R version 4.04. (R Core Team 2021). Multivariate analyses were performed using the R package vegan (Oksanen et al. 2018). We compared the number of experimental days to first appearance of gravid copepods between healthy and infected treatment jars using a paired t-test. Days were averaged between the three copepods within each jar for analysis. We multiplied counts of nauplii, copepodites, non-gravid adults, gravid adults, and total copepods for each subsample by 2 to obtain the number of copepods per jar. We then compared the final numbers of nauplii, copepodites, non-gravid adults, gravid adults, and total copepods of healthy and infected treatment jars with paired t-tests. We finally compared the compositions of developmental stages (as percentages) between treatments using permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) using the eelgrass leaf of origin as strata to account for

pairing. The full set of permutations was enumerated for the permutation tests (n_{perm} = 1023).

We compared the FA profiles between the healthy and infected treatment copepods using PERMANOVA with the eelgrass leaf as strata. We used PERMDISP2 (Anderson 2006) with the same permutational structure to infer whether differences detected in PERMANOVA were driven by location or dispersion. Proportions of four FA were compared between healthy and diseased jar copepods for specific and potential biological indications based on their use as biomarkers (Dalsgaard et al. 2003, Kelly & Scheibling 2012): 1) DHA, as an indicator for Lz or dinoflagellates, 2) EPA, as an indicator of diatoms, 3) palmitoleic acid, as an indicator of diatoms, and 4) ALA, as an indicator of eelgrass being consumed. The comparisons were made using Wilcoxon signed-rank tests.

3. RESULTS

3.1. *Tisbe* consumption of Lz

We directly observed *Tisbe* consuming Lz cells. After moving about the slide, one or few *Tisbe* would settle on a clump of Lz and begin feeding. *Tisbe* would gather small masses of Lz cells, which were then visibly ingested and moved into the gut (Fig. 3.1).

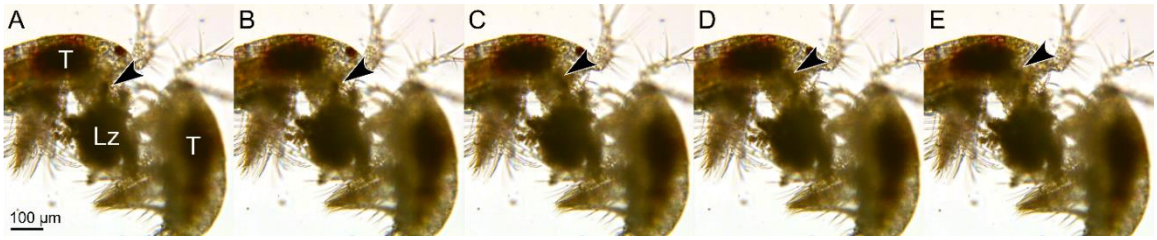

Fig. 3.1. Video image sequence of *Tisbe* copepods feeding on *Labyrinthula zosterae* (Lz) cells. Lz cells were harvested from a culture and provided to the copepods. Arrows point at a mass of Lz cells collected by the copepod and being consumed. T = *Tisbe* copepods, Lz = clump of Lz cells. Images are consecutive still images at 1/30th of a second apart. A levels adjustment with automatic white point was applied in Adobe Photoshop for clarity. An edited (for clarity) video and the unedited video are available upon request.

3.2. Eelgrass wasting disease and *Tisbe* population growth

Eelgrass segments were successfully infected with Lz such that all inoculated segments produced obvious disease lesions that covered nearly or all of each segment. Control segments were visibly healthy and, except for few small lesions on few segments (jars 10, 20), were free of EWD.

There was no difference in the time to first gravid Tisbe between healthy (17 ± 1 days, mean ± sd) and diseased jars (18 ± 2 days, Table 3.1).

Table 3.1. Summary of paired t-tests comparing copepod measures of jars with healthy eelgrass segments and diseased eelgrass segments. Healthy and diseased jar measures are shown as means ± standard deviation. Copepod numbers are graphically presented in Fig. 3.2.

Measure	**Healthy Jars**	**Diseased Jars**	**t =**	**df =**	**p =**
Average days to appearance of first three gravid copepods	17 ± 1	18 ± 2	1.51	9	0.17
Nauplii jar^{-1}	157 ± 109	326 ± 162	3.92	9	0.0035
Copepodites jar^{-1}	293 ± 138	519 ± 195	2.73	9	0.023
Non-gravid adults jar^{-1}	35 ± 11	78 ± 18	5.09	9	0.00066
Gravid adults jar^{-1}	7 ± 6	9 ± 16	0.62	9	0.55
Total copepods jar^{-1}	490 ± 190	933 ± 273	3.80	9	0.0042

The final numbers of nauplii, copepodites, and non-gravid adults jar^{-1} were greater in diseased jars than in healthy jars (Table 3.1, Fig. 3.2). Accordingly, total copepods jar^{-1} was greater in diseased jars than healthy jars (Table 3.1). Numbers of non-gravid adult copepods did not differ between diseased and healthy jars (Table 3.1). Composition of developmental stages did not differ between the treatments (PERMANOVA, pseudo-F = 0.56, df = 1, p = 0.34, n_{perm} = 1023, Fig. 3.3).

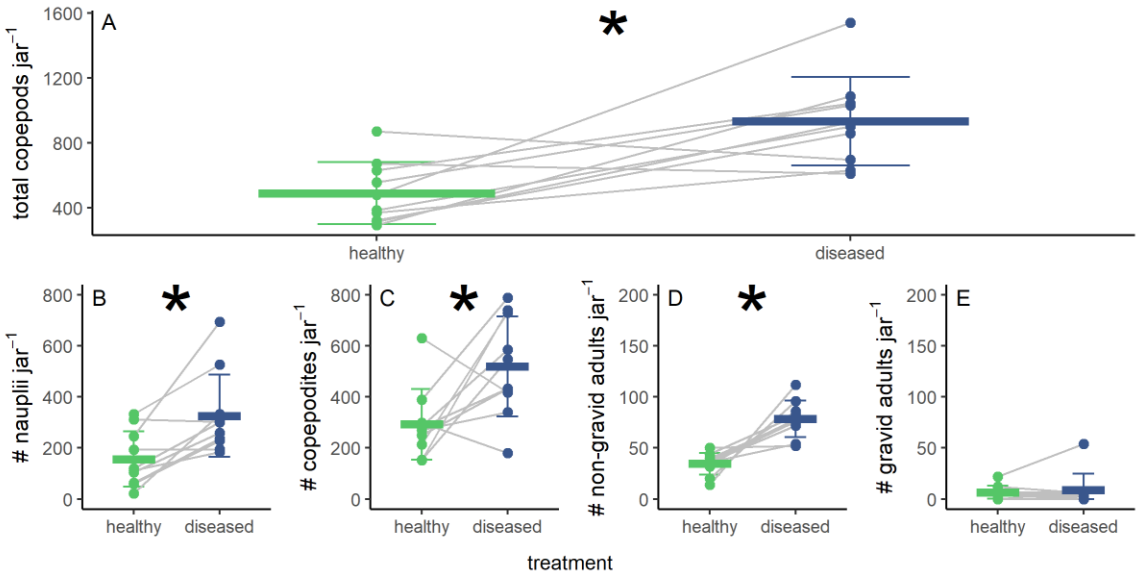

Fig. 3.2. *Tisbe* total copepods (A) and developmental stages (B: nauplii, C: copepodites, D: non-gravid adults, E: gravid adults) jar^{-1} by treatment. Healthy jars contained a control eelgrass segment and diseased jars contained inoculated segments. Jars are represented by individual points; grey lines connect paired jars whose eelgrass segments originate from the same leaves. Heavy horizontal lines indicate means, and error bars are ± 1 standard deviation. Asterisks indicate differences deemed statistically significant through paired t-tests (Table 1). Note differing y-axis ranges of D and E relative to B and C.

3.3. *Tisbe* fatty acids

The fatty acid profiles of copepods raised on diseased eelgrass segments differed from those raised on healthy segments (PERMANOVA, pseudo-F = 2.88, df = 1, p = 0.033, nperm = 1023), despite some visible overlap shown in NMDS (Fig. 3.4). There was no difference in dispersion (PERMDISP2, F = 0.19, p = 0.58, n_{perm} = 1023).

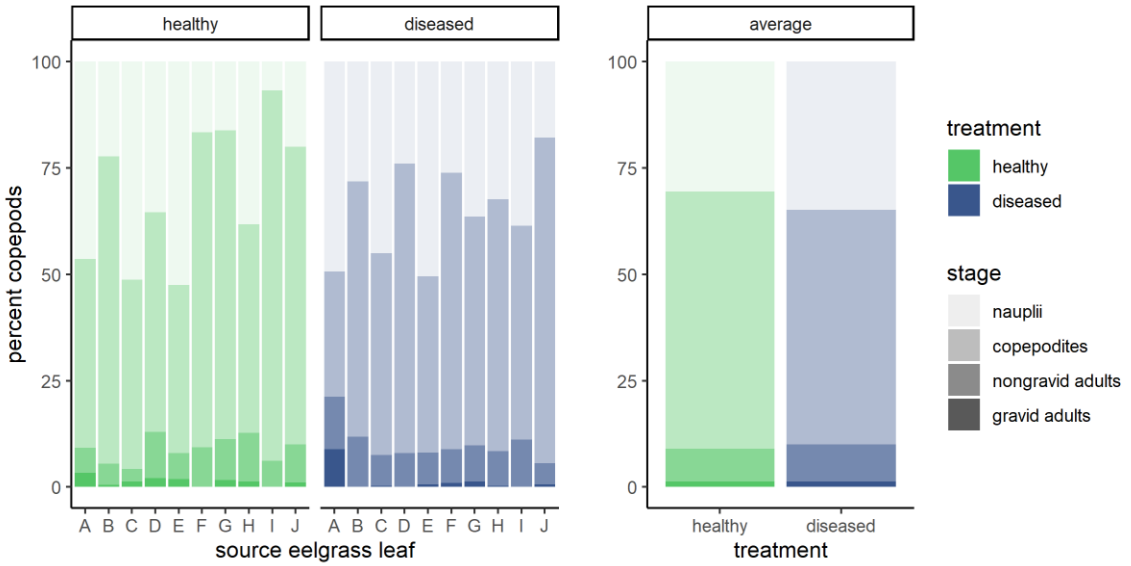

Fig. 3.3. Composition of *Tisbe* developmental stages at end of experiment provided either healthy or diseased eelgrass segments. In the left panel, each stacked bar shows the composition of developmental stages of individual jars, which are paired for sharing the same source eelgrass leaves. The right panel is the average compositions of jars provided either healthy or disease eelgrass segments over the experimental period.

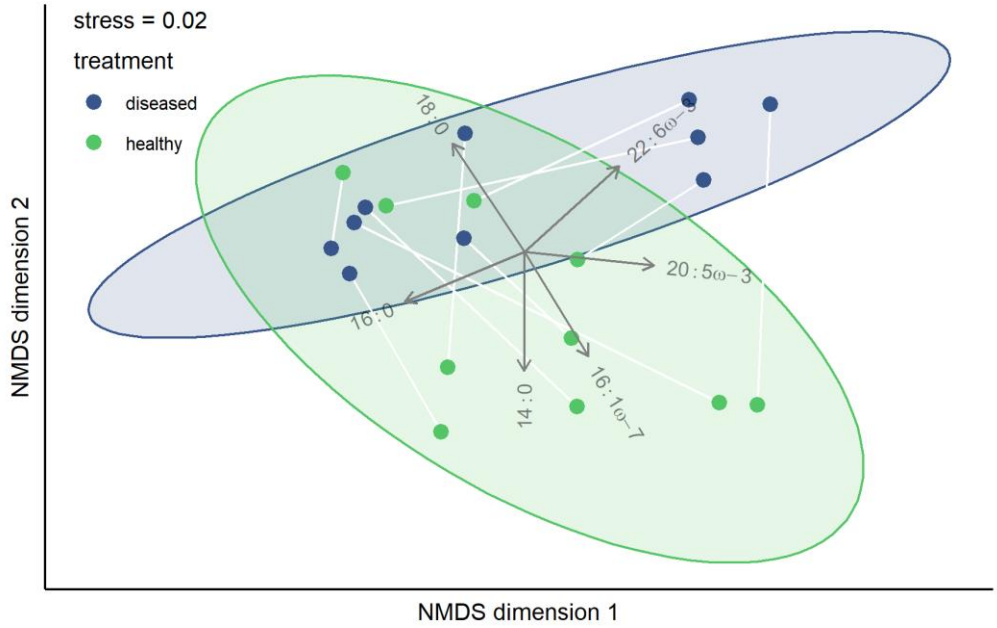

Fig. 3.4. Non-metric multidimensional scaling (NMDS) ordination of proportional fatty acid (FA) compositions of *Tisbe* copepods provided either healthy or diseased eelgrass segments. Ordination was made using FA that compose at least 0.5% of total identified FA. Arrows show relative correlations of FA composing at least 5% of total identified FA with the NMDS dimensions. White lines link individual jars that are paired, being derived from the same eelgrass leaf. Ellipses show standard deviations around the centers of diseased and healthy jars.

For the four FA we compared, only palmitoleic acid was significantly different between copepods from healthy and diseased jars. There was a greater proportion of palmitoleic acid in healthy jar copepods (6.6 ± 8.4%, median ± IQR) compared to diseased jar ones (1.4 ± 2.0%, Wilcoxon signed-rank test, V = 2, p = 0.018). FA proportions did not differ for DHA (healthy: 5.5 ± 6.0%, diseased: 8.0 ± 22.4%, V = 34, p = 0.19), EPA (healthy: 4.9 ± 4.9%, diseased: 3.5 ± 7.7%, V = 20, p = 0.81), nor ALA (healthy: 0.2 ± 0.1%, diseased: 0.1 ± 0.1%, V = 17, p = 0.32).

4. DISCUSSION

Tisbe copepods can consume Lz and be nutritionally supported by diseased eelgrass tissue. Jars provided diseased eelgrass produced about twice the nauplii, copepodites, and non-gravid adults compared to those provided healthy eelgrass. These results suggest that Lz may substantially support secondary productivity through EWD.

We did not ascertain the precise process by which EWD supported *Tisbe* population growth. The most probable and substantial mechanism is that disease liberated nutrients and resources held in eelgrass tissues, which were then accessed by *Tisbe*. This may be either directly by *Tisbe* feeding on the released material or indirectly through *Tisbe*'s consumption of microorganisms that utilized the dissolved nutrients. A similar effect was observed for a chytrid parasite of the freshwater alga *Asterionella*. *Asterionella* is largely inedible for the cladoceran grazer *Daphnia*; however, the chytrid infecting *Astrionella* rendered host biomass edible, as released chytrid spores could be consumed by *Daphnia* (Kagami et al 2007).

Tisbe may also feed upon Lz itself, as *Daphnia* do on chytrid spores. In this study, we directly observed *Tisbe* consuming cultured Lz cells, albeit in an artificial setting with an unnaturally high availability of free Lz cells. Harpacticoid copepods, including *Tisbe*, are known to consume a variety of food items including protists (Vanden Berghe & Bergmans 1981, Parrish et al. 2012). If *Tisbe* indeed consumed and benefited from Lz cells, this could represent a case of trophic upgrading. The production of nutritious DHA by Lz from eelgrass substrates "upgrades" eelgrass's less nutritious FA (Yoshioka et al. 2019). Trophic upgrading has been observed for dinoflagellate consumers of algae preyed upon by copepods (Klein Breteler et al. 1999), chytrid parasites of algae eaten by *Daphnia* (Kagami et al. 2007, Gerphagnon et al. 2019) and flagellate consumers of bacteria grazed by *Daphnia* (Hiltunen et al. 2017).

A logical expectation, and one of our hypotheses, is that *Tisbe* feeding on diseased eelgrass would show FA characteristic of Lz cells. Specifically, a higher content of DHA, which is the primary FA of Lz (Yoshioka et al. 2019), should differentiate the diseased treatment *Tisbe* from the healthy treatment ones. We did not clearly observe this in our study, though four diseased samples were isolated in the direction of DHA in the NMDS (Fig. 3.4). In addition, DHA was not significantly more abundant in diseased treatment copepods relative to healthy ones. Nonetheless, the *Tisbe* FA profiles were different among the treatments, implying an effect of disease on the nutritional quality of *Tisbe*. Another expectation is that, because *Tisbe* derived greater nutrition from the eelgrass, they may show a greater signal of eelgrass FA. In particular, ALA or 18:3ω-3, would be more abundant in the diseased treatment copepods. We did not observe this in our study, which is most likely explained by the decrease of ALA content in eelgrass

tissues through infection (Yoshioka et al. 2019). This highlights a potential issue of using FA to trace primary producers in food webs through disease or even detrital pathways: the most characteristic FA may be lost through decomposition.

Our FA results suggest a difference in the primary or secondary production fostered by either diseased or healthy seagrass. Although the eelgrass segments were wiped and rinsed, the gentle cleaning still allowed diatoms and other microalgae to colonize treatment jars. This was a deliberate strength of this study, as copepods would never be restricted to only eelgrass as a resource *in situ*. In healthy treatments, several samples were separated in the direction of palmitoleic acid (16:1ω-7) and to some extent eicosapentaenoic acid (EPA, 20:5ω-3, Fig. 3.4). Both of those FA are used as biomarkers for diatoms, suggesting that diatoms may have played a role in *Tisbe* nutrition in healthy treatments (Dalsgaard et al. 2003, Kelly & Scheibling 2012). Palmitoleic acid was significantly more abundant in healthy treatment copepods relative to diseased ones, which supports that explanation.

However, EPA did not show the same pattern. This may be expected, though, as animals broadly require both EPA and DHA for growth and reproduction (Parrish 2009), so differences in these FA in copepods might not be detectable unless supplied at significant excess in their diets. While dietary FA does affect the FA found in consumer tissues, zooplankton FA (and those of other organisms) are also constrained by particular biological needs that follow patterns of a consumer's taxonomy (Persson & Vrede 2006, Brett et al. 2009). With such requirements, consumer FA will not exactly reflect those of their food, with differences arising from FA synthesis, modification, or selective retention in the consumer (Galloway & Budge 2020). In particular, *Tisbe* can produce highly

unsaturated FA like EPA and DHA to compensate for the lack of those FA in their diets (Norsker & Støttrup 1994, Nanton & Castell 1998, Parrish et al. 2012), which may have further masked any differences in this study.

It is key to note that copepods were only quantified at the end of the study and it is probable that populations in the jars did not reach an equilibrium state. Additionally, we do not know the trajectories of developmental stages through the experiment and then cannot definitively say whether our counts simply represent an offset in developmental times between treatments. Our results nonetheless strongly suggest a positive effect of EWD on copepod abundance. Firstly, there were ultimately much greater numbers of copepods in the diseased treatment than in the healthy one (Fig. 3.2). There was also no difference in the timing at which gravid copepods first appeared in healthy and diseased jars, which indicates that developmental rate was not substantially different between treatments. An offset in developmental timing would likely produce a pattern in which different developmental stages would have differing patterns of abundance between the treatments. For example, a faster developmental rate would likely leave fewer copepodites in one treatment but greater adults in the same treatment as copepods transitioned from one stage to the next. In our study, though, there were more of each major developmental stage in the diseased treatment than the healthy one. Our results suggest that the difference in population growth was mainly driven by differential survival between treatments, though differences in reproductive rates, because unquantified, cannot be discounted.

Eelgrass segments in our study most simulate detached eelgrass detritus with differing levels of disease. Wave action or boat activity may detach eelgrass leaves (Short

& Wyllie-Echeverria 1996), which through degradation can be made available to detritivores. With detritus, the distinction between Lz as a pathogen or saprotroph (decomposer) is blurred. However, it is likely that a similar pattern would still hold for attached leaves. EWD may also increase detrital production overall, as diseased tissue becomes fragile (R. Yoshioka pers. obs.) and may be detached more easily. Older, senescent leaves are also more susceptible to EWD (Groner et al. 2014) and their degradation may be hastened through infection. Through these combined effects, EWD likely makes eelgrass biomass more available in total to copepod consumption.

A logical next step of EWD increasing detritivore resources is that detritivores should become more abundant for their consumers. An increase in copepods should likewise benefit various predators that prey upon them, such as developing fish (Sibert et al. 1977, Tipton & Bell 1988, Jenkins et al. 2011). Future research should verify whether EWD may be related to the abundance of harpacticoid copepods in the field. If our study's pattern holds, we would expect an increase in copepod abundance with EWD levels (disease severity or prevalence) until a maximum, where high disease reduces eelgrass populations such that biomass or habitat are reduced for the copepods. Predators of copepods may follow the same pattern, though a correlation may be less pronounced with predators one trophic level more distant to EWD, leaving other ecological factors to affect this relationship. It should be emphasized that, in any case, potential benefit provided by EWD at very high disease levels is likely outweighed by the destruction of seagrass habitat.

In our study, we have expanded the potential ecological role of a parasite beyond the contexts in which it is most considered. While we highlight that future work should

examine the destructive effects of Lz, especially with the importance of seagrass ecosystems (Nordlund et al. 2016, Unsworth et al. 2019) and their global threats (Orth et al. 2006, Waycott et al. 2009), we also emphasize that ecologists should consider *Labyrinthula*'s nutritive effects and EWD's potential contributions to secondary production.

BRIDGE

In Chapter 3, I found that EWD facilitated the population growth of *Tisbe* copepods. These results, while compelling, were caused by a microparasite and isolated to an experimental, laboratory setting. In Chapter 4, I consider another study system, this time based on field-collected data and a macroparasite. Studying the rhizocephalan parasite *Sylon hippolytes* of the shrimp *Pandalus danae*, I asked whether *Sylon* induces changes that could affect trophic relationships of its host.

CHAPTER IV

A RHIZOCEPHALAN PARASITE INDUCES PERVASIVE EFFECTS ON ITS SHRIMP HOST

This work includes coauthors Suhn Brown, for in-progress work on behavioral changes in sylonized shrimp, Nancy Treneman, for algal epibiont identification, Dr. Julie Schram, for assistance in FA analysis, and Dr. Aaron Galloway, as principal investigator. All authors will ultimately contribute to the manuscript. I performed all field data collection and sampling, FA extractions, data analyses, and writing to date.

1. INTRODUCTION

Parasites are increasingly recognized for their diverse and substantial roles in their ecosystems (Preston et al. 2016). These can include population regulation or mass mortality of their hosts, which can cascade to other ecosystem members. Further, parasites can affect the properties of their hosts and then influence the organisms that interact with their hosts. Termed trait-mediated indirect interactions (TMII), such effects are less reported in the literature than numeric effects (density-mediated indirect interactions or DMII, Preston et al. 2016) but can still dramatically impact ecosystems (e.g., Wood et al. 2007, Hernandez & Sukhdeo 2008, Sato et al. 2012). With parasites' diverse and consequential effects, a holistic understanding of ecosystems requires measurement and recognition of their potential influences (Gehman et al. 2019).

Rhizocephalan barnacles are particularly fascinating in their adaptations to their parasitic lifestyle and their well-known capacity to affect their ecosystems through both density- and trait-mediated indirect interactions (Bishop & Cannon 1979, Høeg 1995, Mouritsen & Jensen 2006). Evolving from a filter-feeding ancestor, the Rhizocephala infect crustaceans, most commonly decapods (Høeg 1995, Glenner & Hebsgaard 2006, Ewers-Saucedo et al. 2019). They have adapted to a parasitic lifestyle by losing typical adult barnacle features and a having a root-like, trophic internal structure (interna) and a bulbous, reproductive external structure (externa) (Høeg 1995). As a classical parasitic castrator, rhizocephalans may regulate host populations by limiting reproduction (Kuris & Lafferty 1992, Torchin et al. 2001, but see Innocenti & Galil 2011a) and also alter host ecological function through behavioral and physiological changes (Smith 1911, Bishop & Cannon 1979, Mouritsen & Jensen 2006).

These latter effects, or TMIIs, are well documented in relationships between brachyuran hosts and rhizocephalans. The green crab *Carcinus maenas* (Linnaeus) infected by *Sacculina carcini* Thompson burrow less than uninfected conspecifics (Mouritsen & Jensen 2006). Burrowing behavior scours away epibiotic organisms on the crabs' surface, and, with the behavior reduced, an infected crab becomes a hard substrate for sessile organisms in an otherwise soft-sediment habitat (Mouritsen & Jensen 2006). Parasite-associated epibiotic fouling has also been observed in other crab-rhizocephalan relationships, including *Portunus sanguinolentus* (Herbst)-*Diplothylacus sinensis* (Keppen) (Yang et al. 2014), *Charybdis longicollis-Heterosaccus dollfusi* (Innocenti & Galil 2011b), and *Portunus pelagicus* (Linnaeus)-*Sacculina granifera* Boschma (Gaddes & Sumpton 2004). The patterns are generally attributed to changes in host behavior and

the inhibition or cessation of molting, a common effect of rhizocephalans (Phillips & Cannon 1978, Bishop & Cannon 1979, Høeg 1995, Mouritsen & Jensen 2006).

In contrast, the influence of rhizocephalans on epibiotic fouling is to our knowledge not yet described for shrimp hosts. Relative to carcinized or crab-like decapod body forms, fouling may be more detrimental for caridoid or shrimp-like forms. Caridoid decapods, formerly classified as Natantia, often rely on swimming or a tail-flip escape for locomotion and defense, which may be impaired by epibiont-produced drag (Bauer 1978, 1981). This difference is suggested to explain why specialized grooming behaviors are less common in crabs than shrimp (Bauer 1975, 1981), although crabs may rely on other anti-fouling defenses or be more tolerant of fouling (Fernandez-Leborans 2010). If rhizocephalans affect fouling in shrimp, they in turn could affect other organisms by providing substrate for epibionts and, through impairing escape responses, increasing the predation success on the shrimp.

Altered predation rates could be consequential, as shrimp are crucial prey items for marine organisms. One approach to study trophic relationships and nutrition is through fatty acid (FA) analyses, which exploit the conserved nature of FA in organismal lipids to trace dietary interactions (Dalsgaard et al. 2003, Kelly & Scheibling 2012). In addition, nutritionally valuable fatty acids such as ω-3 polyunsaturated FA (PUFA) can be identified. Several PUFA are vital in growth and reproduction in marine organisms (Parrish 2009, Winder et al. 2017), and shrimp resources allocated to reproduction are likely diverted to rhizocephalans as parasitic castrators. Differences in nutritious PUFA such as EPA and DHA between parasitized and uninfected shrimp could indicate whether parasitism improves or reduces a shrimp's quality as a food source. Thus, investigating

the FA of infected shrimp may provide insight to the nutrition of the shrimp for its predators and the provisioning of host resources.

Changes in lipid provisioning have been observed in rhizocephalan infections of crabs. In *Carcinus maenas* and *Inachus* sp., *Sacculina* parasitism is associated with the development of fat in the hepatopancreas and blood that are typical for female crabs with maturing ovaries, suggesting feminizing effect on lipid metabolism (Smith 1911). This is related to a famous morphological and physiological effect of rhizocephalans: host feminization. Rhizocephalan parasites of brachyuran and anomuran crabs are documented to induce female morphological features in infected male crabs (Høeg 1995). For example, wide pleons are secondary sexual characters of female crabs, and infected male crabs often show a broadening of the pleon relative to their uninfected male counterparts (Høeg & Lützen 1995, Høeg 1995, Kristensen et al. 2012). Rhizocephalans can also affect crab behavior, inducing behaviors typical of ovigerous female crabs and result in host care of the parasite's externa (Phillips & Cannon 1978, Ritchie & Høeg 1981, Høeg & Lützen 1995). There is scant but suggestive evidence for feminizing effects in shrimps. Lützen (1981) observed two male *Spirontocaris lilljeborgi* (Danielssen) shrimp infected by *Sylon hippolytes* M. Sars with morphologies of pleopod 1 between those of male and female shrimp; in one, the gonad appeared to hold both spermatogonia and oocytes. In *Pandalus platyceros* J.F. Brandt, *Sylon*-infected young (non-female) shrimp showed gonad and secondary sex characteristic abnormalities (Bower & Boutillier 1990a).

Shrimp of the genus *Pandalus* are widespread and ecologically important (Bergström 2000). In the northeastern Pacific Ocean, they have a variety of predators including rockfish, cod, octopus, and dogfish (Butler 1980, Albers & Anderson 1985,

Bergström 2000, Turner et al. 2017). Species such as *P. platyceros*, *P. jordani* (Balss), *P. eous* Makarov, and *P. danae* Stimpson are caught commercially and/or recreationally (Bergström 2000). The rhizocephalan *S. hippolytes* infects these and other shrimp species in the region and can reach high prevalences (over 32%, in Bower & Boutillier 1990). Globally, pandalid shrimp are only second to penaeid shrimp in terms of their commercial importance (Holthuis 1980). With the ecological and economic importance of these shrimp, it is especially valuable to understand the potential effects of *Sylon* on pandalid shrimp. Additionally, pandalid shrimp offer an interesting case study for rhizocephalan-induced feminization. Several pandalids are protandric hermaphrodites (i.e., developing from male to female), while most decapods are gonochoric (Bergström 2000, Chiba 2007). Most of the relatively few (85) species of hermaphroditic decapods are shrimp (Chiba 2007). This begs the question of whether and how *Sylon* may feminize shrimp already destined to become female.

Here, we describe effects of *Sylon hippolytes* parasitism in the dock shrimp *Pandalus danae* (Fig. 4.1) in a high-prevalence population found at Friday Harbor Laboratories (FHL, University of Washington, Friday Harbor, Washington, USA). We surveyed the population to determine *Sylon* prevalence and the association of epibiotic fouling and parasitism. We measure and describe potential lipid and FA changes in the *P. danae* caused by *Sylon* infection. Finally, we extend our results to estimate the effects of parasite-altered FA on dietary analysis, using rockfish as a model.

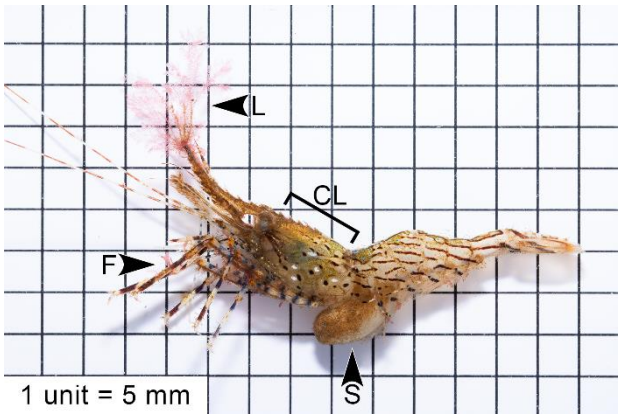

Fig. 4.1. *Pandalus danae* infected by *Sylon hippolytes*. Infection at this stage is apparent with a conspicuous externa (S) emerging from the pleon of the shrimp. Post-orbital carapace length (CL) was measured from the orbit to the dorsal edge of the carapace. Epibiotic organisms including "lacy red algae" (L) and "flat red algae" (F) are visible. *Ephelota* is also present but minute.

2. MATERIALS AND METHODS

We performed all data analyses in R version 4.0.4 (R Core Team 2021). All work was conducted under institutional approval and guidelines for COVID-19 safe research practice.

2.1. Study location and specimen collections

We collected *P. danae* from the docks at FHL in July and August 2020 under the general FHL scientific take permit, which is overseen by the FHL Director in accordance with local regulations. We used a large custom dip net made of ~150-μm bolting cloth to catch shrimp from pilings; a flexible, semicircular edge on the net allowed us to more completely capture shrimp on pilings and avoid bias in collection. After capture, shrimp were held in flow-through seawater tables of the laboratories.

2.2. *Sylon* prevalence and epibiosis

We collected and measured 575 shrimp in daily batches on July 14-20 and 22, 2020 To prevent duplicate counting of shrimp, we held all measured shrimp in a separate sea table until measurements ended on July 22, after which all measured shrimp were released. To facilitate handling, we narcotized shrimp by slowly cooling them in a bag of seawater set in a cooler of ice or ice packs. We monitored shrimp scaphognathites (bailers) as a proxy for respiration to avoid euthanizing shrimp (incidental mortality was 2.6%). For each shrimp, we measured the post-orbital carapace length (hereon "size") as a proxy for age using digital calipers (Fig. 4.1). The sex of each shrimp (juvenile, male, transitional, and female) was scored using the features of the first pleopods and sternal spines. We recorded the presence or absence of any macroscopically visible epibionts: the suctorian ciliate *Epelota* sp., algae (separated into morphological groups), spirorbid worms, bryozoans, and hydroids. Organisms that were macroscopically indistinguishable or whose presence was ambiguous (e.g. diatoms) were not recorded. We scored *Sylon* presence and stage visually and nonlethally by observing through the transparent, ventral cuticle of the pleon. Stages were determined as 1) "uninfected", showing no signs of *Sylon*, 2) "interna only", showing milky, web-like growths under the pleon but no other signs of *Sylon*, 3) "primordial externa", a nucleus or developing externa that has not emerged from the pleon, 4) "present", an emerged externa, and 5) "scar", where the externa has fallen away leaving a melanized scar. For shrimp with *Sylon* externa(e), we also measured the externa width. To facilitate later identification of epibiotic algae, we sampled algae from several shrimp and mounted them on slides using glycerol jelly (Dioni 2003). Shrimp measurement items were informed by an independent preliminary

survey in August 2019 (N = 313 shrimp), which lacked sex and *Sylon* stage data (externa or scar presence/absence only). A sample size of over 500 shrimp was targeted for the 2020 survey so that prevalence measurements had a precision of well below 1%.

We modeled the association between size and *Sylon* stage and epibiosis separately for *Ephelota*, small red algae, lacy red algae, and flat red algae using logistic regression models (N = 575 shrimp). Size was included as a predictor because larger shrimps were expected to have longer intermolt periods (Daoud et al. 2010) and provide a larger settlement surface for epibionts, increasing the probability of epibiosis. We did not model other epibionts, which had very low prevalences, because patterns of these rare events were likely not captured accurately with our sample size.

2.3. Sexual effects of sylonization

We investigated whether shrimp sex was associated with sylonization by modelling sex predicted by size and *Sylon* presence, fitting the data using continuation ratio models with the R package VGAM (Yee 2021). We omitted juvenile shrimp from this analysis as no sylonized juveniles were observed. Two un-sexed shrimp were also omitted from analysis (N = 564 shrimp). Proportional odds ordinal logistic regression was deemed inappropriate because of violations of the parallel-lines assumption (McCullagh 1980, Harrell Jr 2015).

2.4. Fatty acids of sylonized shrimp

We collected 22 each of sylonized and uninfected shrimp on July 28, 29, and August 2, 3, 2020. We chose sylonized shrimp with relatively large, well-developed

externae and then selected uninfected shrimp of similar sizes to limit size or sex effects. We held shrimp in sea tables without feeding for at least 24 hours before preparing them for FA sampling. Shrimp were euthanized by slowly cooling them to narcotize as described above and then transferring to a -20 ˚C freezer until shrimp were completely unresponsive and scaphognathite movement ceased. We did not use chemical anesthetics for euthanasia because of the potential to contaminate samples (especially clove oil).

We sampled 12 each whole sylonized and whole uninfected shrimp. We removed the pereiopods, third maxillipeds, and antennal flagella before dipping shrimp in fresh tap water and freezing at -80 ˚C. The limbs were removed to reduce debris in the later lipid extraction process. The freshwater dip removed excess salt that could interfere with later weighing steps prior to lipid extraction.

We dissected 10 each of sylonized and uninfected shrimp. For both, we dissected and stored abdominal muscle tissue and the hepatopancreas. For sylonized shrimp, we also removed and stored the externae. Dissected tissues were frozen at -80 ˚C.

We extracted lipids from frozen samples following modified methods of Taipale et al. (2013, 2016). Briefly, we freeze dried and ground the samples before adding 2:1 chloroform:methanol to a weighed amount of ground sample. We then added an internal C19:0 standard and 0.9% aqueous NaCl. The samples were vortexed and sonicated, after which the chloroform layer was removed as the lipid extract. We added additional chloroform and repeated the vortexing/sonicating with the remaining layer, pooling the two chloroform lipid extracts within samples. We measured lipid content gravimetrically from the lipid extract.

We transesterified lipid extracts to derive fatty acid methyl esters (FAMEs) prior to analysis using a QP2020 Shimadzu gas chromotographer-mass spectrometer (GC-MS). For transesterifaction, we evaporated chloroform from lipid extracts and re-dissolved lipids in toluene. We added 1% sulfuric acid in methanol and incubated the samples at 90 ˚C for 90 minutes. The sulfuric acid was neutralized with 2% $KHCO_3$ and hexanes were added. We vortexed and centrifuged the samples and then removed and stored the hexanes layer containing FAMEs, repeating hexanes extraction of FAMEs once. All lipid extraction and transesterification steps were performed under a nitrogen gas atmosphere. GC-MS analysis and FA identification were as in Yoshioka et al. (2019) and Taipale et al. (2013, 2016).

We visualized the multivariate FA data using non-metric multidimensional scaling (NMDS) and compared FA profiles among tissues and *Sylon* status using permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) 9999 permutations. For dissected tissues, we omitted externae and compared the remaining dissected tissues. We accounted for tissues belonging to the same shrimp by using shrimp identity as a stratum. We then compared *Sylon* status within tissues as a post hoc test, penalizing alpha by dividing it by 2 (= 0.025), the number of additional comparisons. Multivariate analyses were performed in the vegan package (Oksanen et al. 2018) with Euclidean distances. We limited FA analyses to FA that composed at least 0.5% of the total identified FA in any *Sylon*-tissue combination on average. We used PERMDISP2 (Anderson 2006) to infer whether significant PERMANOVA results were due to differences in dispersion or location. We compared lipid content between sylonized and uninfected shrimp for each tissue type using Wilcoxon rank-sum tests.

2.5. QFASA diet analysis including sylonized shrimp

To investigate how sylonization might affect dietary analysis studies, we performed a mixing model exercise using Quantitative Fatty Acid Signature Analysis or QFASA (Iverson et al. 2004), implemented in the R package qfasar (Bromaghin 2017). Using literature FA profiles of a fish predator and other potential prey, we tested how including *Sylon* parasitism in shrimp as a prey type affected consumer diet predictions of QFASA. We used the copper rockfish *Sebastes caurinus* Richardson as our model predator, which has been documented to feed heavily on *P. danae* (Turner et al. 2017). We selected five prey species based on stomach contents found and discussed by Turner et al. (2017) and literature FA data availability: sand lance (*Ammodytes hexapterus* Pallas), herring (*Clupea pallasii* Valenciennes), pygmy rock crab (*Glebocarcinus oregonensis* (Dana)), Dungeness crab (*Metacarcinus magister* (Dana)), and *P. danae*. These species covered the three major groups of prey (fish, crabs, and shrimp) found by Turner et al. (2017) and, except for *M. magister*, had literature values available from samples in the Salish Sea, where this study's work was conducted. The predator and prey data are described in Table 4.1.

To account for consumer FA modification (i.e. changes in FA composition as a consumer incorporates dietary FA into its tissues) in our mixing models (Galloway & Budge 2020), we transformed prey FA signatures into predator space using the regression fits described in Jardine et al. (2020) of prey FA to Actinopterygii predator FA. The regressions were distinct for each FA and made calibration coefficients, which typically account for trophic fractionation in the QFASA approach (Iverson et al. 2004), unnecessary (i.e., all calibration coefficients were set to 1). We used 19 FA as tracers,

based on the set used by Jardine et al. (2020) and on data availability: 14:0, 15:0, 16:0, 17:0, 20:0, 24:0, 16:1ω-7, 18:1ω-9, 18:1ω-7, 20:1ω-9, 24:1ω-9, 18:2ω-6, 18:3ω-3, 18:4ω-3, 20:3ω-3, 20:4ω-6, 20:5ω-3, 22:5ω-3, and 22:6ω-3. For all QFASA models, we used the Kullback–Leibler distance measure and an augmented proportion.

Table 4.1. Consumer and prey species used in the hypothetical Quantitative Fatty Acid Signature Analysis (QFASA) exercise of copper rockfish diet dependent on shrimp sylonization. In round 1, sylonized and uninfected shrimp were treated as separate items. In round 2, shrimp were combined and treated as a single prey item. In round 3, sylonized and uninfected shrimp were combined, but different proportions of sylonized and uninfected shrimp were sampled to manipulate the importance of *Sylon* in the shrimp prey type.

Species	**Round 1**	**Round 2**	**Round 3**	**Location**	**Data Reference**
Copper rockfish, *Sebastes caurinus*	Consumer, N = 12			San Juan Islands, Washington, USA	(Bromaghin et al. 2013)
Pacific sand lance, *Ammodytes hexapterus*		N = 12			
Pacific herring, *Clupea pallasii*		N = 24			
Pygmy rock crab, Glebocarcinus oregonensis		N = 29		San Juan Islands, Washington, USA	(Galloway et al. 2013)
Dungeness crab, *Metacarcinus magister*		N = 24		Charleston, Oregon, USA	(Thomas et al. 2020)
Sylonized *P. danae*	N = 12	N = 24	N = 12 N_{sylon} = 0 -12 N_{uninf} = 12 - 0	Friday Harbor, San Juan Island, Washington, USA	This study
Uninfected *P. danae*	N = 12				

We performed three rounds of QFASA: 1) with sylonized and uninfected shrimp as separate prey types, 2) with sylonized and uninfected shrimp combined as a single prey type, and 3) with sylonized shrimp and uninfected shrimp combined as a single prey type, but varying contributions of sylonized shrimp. In rounds 1 and 2, all *P. danae* data were used to predict rockfish diets. Prediction uncertainty was obtained as standard errors by bootstrap sampling prey profiles in 100 iterations. In round 3, we subsampled our *P. danae* data (without replacement) to create sets of 12 shrimp, drawing different numbers

of sylonized and uninfected shrimp to manipulate proportions of sylonized shrimp. For each proportion (1/12 to 11/12 shrimp), we sampled 100 sets and ran QFASA 100 times to obtain average rockfish diet predictions. Uncertainty was obtained as standard deviations of the mean predictions within each proportion set. Proportion sets 0/12 and 12/12 used the single estimates of using only uninfected and only sylonized shrimp. No variation was obtained for those sets because, without replacement, the same sets would be repeatedly sampled.

3. RESULTS

3.1. *Sylon* prevalence and epibiosis

We measured 575 shrimp, which ranged in size (carapace length) from 5.52 to 26.17 mm (mean ± SE, 13.62 ± 0.11 mm). We scored 9 (1.6%) shrimp as juvenile, 404 (70.3%) as male, 54 (9.4%) as transitional, and 106 (18.4%) as female. Two shrimp were accidentally moved into a communal post-measurement tank before scoring sex, so those individuals lack sex data. *Sylon* prevalence was 42.8% overall (246 shrimp), with 10.4% (60) shrimp with only an interna, 15.1% (87) with a primordial externa, 13.2% (76) with an emerged externa, and 4.0% (23) with scars.

Ephelota was the most common epibiont, found on 397 (69.0%) of all shrimp. “Small” red algae were found on 257 (44.7%) of shrimp, “lacy” red algae on 93 (16.2%) shrimp, “flat” red algae on 21 (3.7%), “filamentous” red algae on 8 (1.4%), and “stringy brown” algae on 20 (3.5%). Animal epibionts were rare but included spirorbid worms (2 shrimp), bryozoans (2), and hydroids (1). Thoracican barnacles were not observed in our 2020 survey described here, but one was found on a single infected shrimp in 2019

(Yoshioka, unpublished data). Algal morphological groups represented several algal species (Table 4.2). One species, *Scagelia occidentalis*, was also found as an epiphyte on brown and red algae collected from the FHL docks.

Table 4.2. Algal morphological groups assessed in survey and corresponding species identified using sampled, slide-mounted epibionts.

Morphological Group	**Description**	**Species**
Small Red Algae	Minute red algae with no macroscopically visible features apart from color and presence	Small individuals of other species
Lacy Red Algae	Branching and feather-like red algae	*Antithamnion kylinii* N.L.Gardner, 1927; *Callithamnion acutum* Kylin, 1925; *Scagelia occidentalis* (Kylin) Wollaston, 1972
Flat Red Algae	Foliose or blade-like red algae	*Nitophyllum hollenbergii* (Kylin) I.A.Abbott, 1969
Filamentous Red Algae	Small, filiform red algae with no to few branches	*Polyostea robusta* (N.L. Gardner) Savoie & GW Saunders, 2016
Stringy Brown Algae	Olive-brown filamentous algae	*Ectocarpus commensalis* Setchell & N.L.Gardner, 1922

For *Ephelota*, small red algae, lacy red algae, and flat red algae, rates of epibiosis increased with *Sylon* stage (Fig. 4.2). We did not analyze other epibionts. Logistic regression models with the lowest AIC and BIC values included only *Sylon* stage as a predictor for *Ephelota* and lacy red algae (Fig. 4.2A, C, Table 4.3). The model using size and *Sylon* stage had the lowest AIC and BIC values for small red algae (Fig. 4.2F, Table 4.3). The lowest AIC model for flat red algae included size and *Sylon* stage (Fig. 4.2H), but the lowest BIC model used *Sylon* stage only (Fig. 4.2D, Table 4.3).

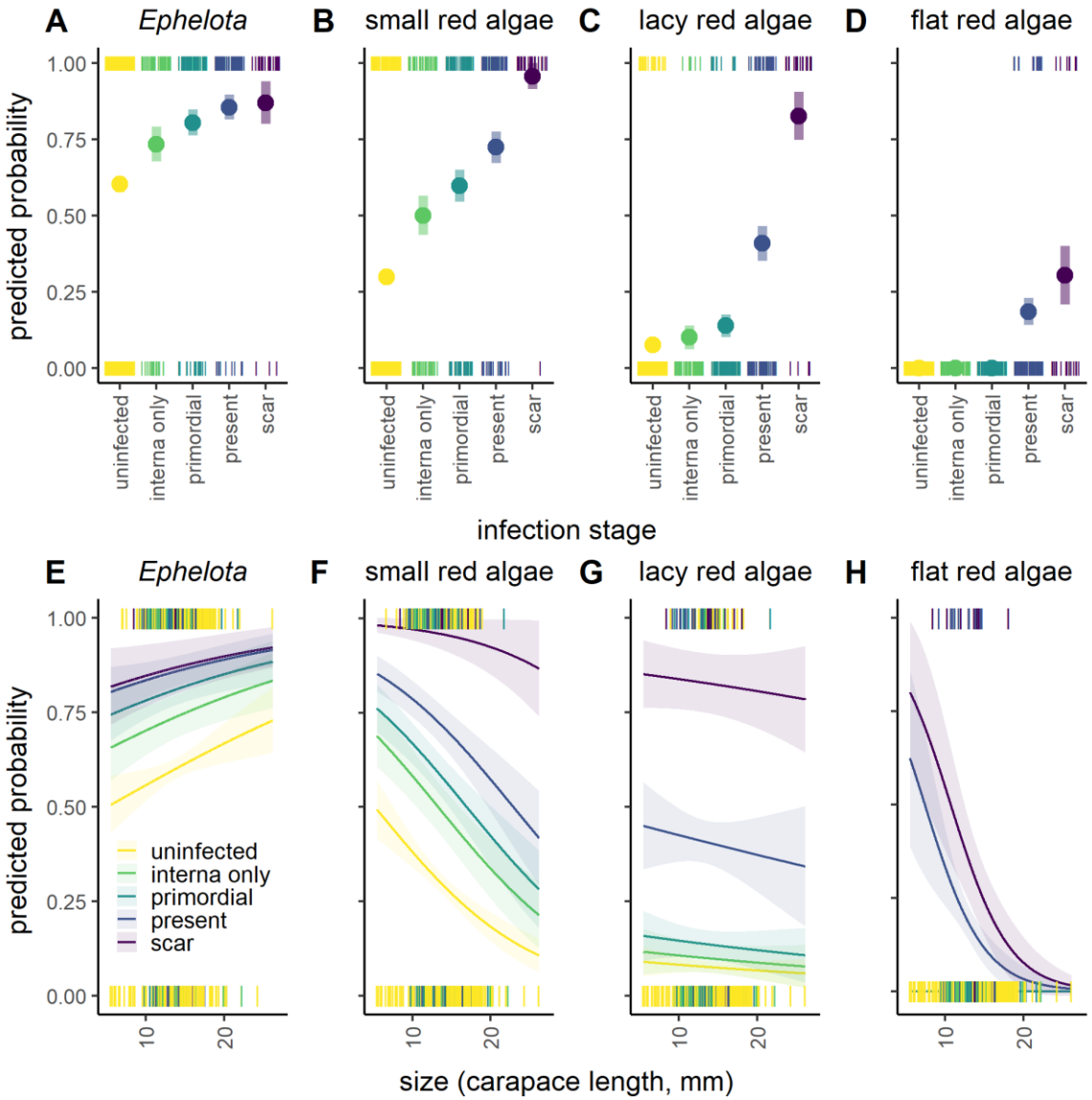

Fig. 4.2. Predictions of logistic regression models of the probability of epibionts on shrimp by *Sylon* infection stage (A-D) or size and infection stage (E-H). Epibionts are (A, E) *Ephelota*, (B, F) small red algae, (C, G) lacy red algae, and (D, H) flat red algae. Rug plots at 0 and 1 are recorded absence and presence, respectively, of the epibiont on surveyed shrimp. Points are logistic regression predictions with ± 1 standard errors. Curves show model predictions with ± 1 standard errors as shading.

Table 4.3. Corrected Akaike's (AICc) and Bayesian Information Criteria (BIC) values for logistic regression models of epibiotic fouling of shrimp based on size (postorbital carapace length) and/or *Sylon* stage. ΔAICc and ΔBIC are the differences from lowest model AICc and BIC, respectively. AICc weights are *w*. Models with the lowest AICc or BIC values are bolded. We present predictions of *Sylon* and Size + *Sylon* models in Fig. 4.2.

	Model	**AICc**	**ΔAIC**	**BIC**	**ΔBIC**	***w***
Ephelota	*Ephelota* ~ 1 (null)	713.6	24.9	717.9	7.6	<0.0001
	Ephelota ~ Size	714.9	26.3	723.6	13.3	<0.0001
	Ephelota* ~ *Sylon	688.7	-	710.3	-	0.48
	Ephelota ~ Size + *Sylon*	688.8	0.1	714.7	4.4	0.46
	Ephelota ~ Size + *Sylon* + Size:*Sylon*	692.8	4.2	736	25.7	0.06
"Small" Red Algae	"Small" red algae ~ 1 (null)	792.6	90.2	797	68.6	<0.0001
	"Small" red algae ~ Size	782.5	80.1	791.2	62.8	<0.0001
	"Small" red algae ~ *Sylon*	709.1	6.7	730.8	2.4	0.03
	"Small" red algae ~ Size + *Sylon*	702.4	-	728.4	-	0.89
	"Small" red algae ~ Size + *Sylon* + Size:*Sylon*	707.4	4.9	750.5	22.1	0.08
"Lacy" Red Algae	"Lacy" red algae ~ 1 (null)	510.9	91.1	515.3	73.8	<0.0001
	"Lacy" red algae ~ Size	512.6	92.8	521.3	79.8	<0.0001
	"Lacy" red algae ~ *Sylon*	419.9	-	441.5	-	0.65
	"Lacy" red algae ~ Size + *Sylon*	421.7	1.9	447.7	6.2	0.25
	"Lacy" red algae ~ Size + *Sylon* + Size:*Sylon*	423.6	3.7	466.7	25.2	0.10
"Flat" Red Algae	"Flat" red algae ~ 1 (null)	182.2	73.5	186.6	53.9	<0.0001
	"Flat" red algae ~ Size	181.9	73.2	190.6	58	<0.0001
	"Flat" red algae ~ *Sylon*	111	2.3	132.7	-	0.24
	"Flat" red algae ~ Size + *Sylon*	108.7	-	134.7	2.1	0.74
	"Flat" red algae ~ Size + *Sylon* + Size:*Sylon*	116.7	8	159.9	27.2	0.01

3.2. Sexual effects of sylonization

We observed 9 juvenile shrimp, none of which were sylonized. Uninfected shrimp were 68.4% (225 shrimp) male, 6.7% (22) transitional, and 22.2% (73) female. Sylonized shrimp were 72.8% (179 shrimp) male, 13.0% (32) transitional, and 13.4% (33) female. The two un-sexed shrimp were both sylonized.

The continuation ratio model predicting sex including size, *Sylon* presence, and their interaction had the lowest AICc and BIC values of the relevant models (Table 4.4). The model predicted a greater probability of being transitional or female at a smaller size for sylonized shrimp compared to uninfected shrimp (Fig. 4.3). The inverse was also

predicted; the probability of a shrimp being transitional or male was also greater at larger sizes for sylonized shrimp compared to uninfected shrimp (Fig. 4.3).

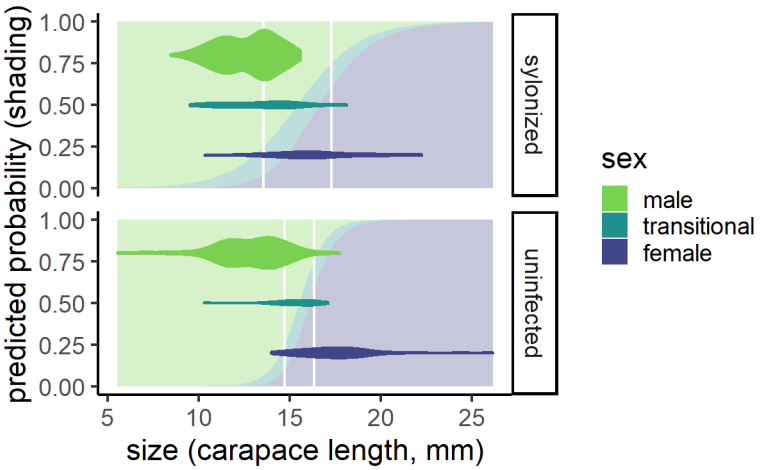

Fig. 4.3. Shrimp size distributions (violins) and continuation ratio model predictions (shading) for shrimp sex by *Sylon* infection status. Violin plots are scaled to counts *within* infection status and are vertically dodged for visibility (i.e., vertical locations are meaningless with respect to y-axis). Stacked, shaded areas show the predicted probabilities of a shrimp at a given size being male, transitional, or female according to the morphology of pleopod 1 and sternal spines. White vertical lines indicate size thresholds at which 25 and 75% of the shrimp are either transitional and female based on model predictions.

Table 4.4. Corrected Akaike's (AICc) and Bayesian Information Criteria (BIC) values for continuation ratio models of shrimp sex based on size (carapace length) and *Sylon* presence. ΔAICc and ΔBIC are the differences from lowest model AICc and BIC values, respectively. AICc weights are *w*. Predictions for the lowest AICc and BIC model are shown in Fig. 3.

Model	**AICc**	**ΔAICc**	**BIC**	**ΔBIC**	***w***
Sex ~ 1 (null)	881.4	328.4	890	302.6	<0.0001
Sex ~ Size	574	21	591.3	3.9	<0.0001
Sex ~ *Sylon*	873.1	320.1	890.4	303	<0.0001
Sex ~ Size + *Sylon*	569.4	16.5	595.3	7.9	0.0003
Sex ~ Size + *Sylon* + Size:*Sylon*	553	-	587.4	-	0.9997

3.3. Fatty acids of sylonized shrimp

We identified 57 FA in our samples, of which 35 FA were present at 0.5% of total identified FA (TFA) or greater. Seven FA were present at 5% TFA or greater: palmitic acid (16:0), palmitoleic acid (16:1ω-7), stearic acid (18:0), cis-vaccenic acid (18:1ω-7),

oleic acid (18:1ω-9), eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acid (DHA, 22:6ω-3). In whole shrimp, EPA and palmitic acid were the most abundant FA in both sylonized and uninfected whole shrimp (mean ± sd, EPA: 21.0 ± 1.3%, 20.7 ± 1.9 %, resp.; palmitic: 15.7 ± 0.5 %, 14.9 ± 0.7%, resp.). Oleic acid was the third most abundant FA in whole sylonized shrimp (14.9 ± 1.3%), whereas DHA was the third most abundant in uninfected ones (12.6 ± 1.6%). In both sylonized and uninfected hepatopancreases, EPA (17.0 ± 3.9%, 15.1 ± 3.8%, resp.), palmitic acid (13.0 ± 1.8%, 12.5 ± 0.9%, resp.), and oleic acid (10.0 ± 4.8%, 8.5 ± 5.0%, resp.) were the most abundant FA. In sylonized and uninfected abdomens, the most abundant FA were EPA (20.9 ± 1.8%, 21.5 ± 1.3%, resp.), palmitic acid (18.8 ± 1.3%, 18.7 ± 0.7%, resp.), and DHA (15.9 ± 1.0%, 15.7 ± 1.1%, resp.). *Sylon* externae were dominated by oleic acid (22.6 ± 2.0%), followed by EPA (19.7 ± 1.8%) and palmitic acid (16.6 ± 0.9%).

FA profiles were distinct among sylonized and uninfected whole shrimp (PERMANOVA, Table 4.5, Fig. 4.4B). In dissected shrimp, FA profiles differed between sylonized shrimp and uninfected shrimp and by tissue, but their interaction was not significant (Table 4.5, Fig. 4.4C, D). Within tissues, however, differences between sylonized and uninfected tissues were non-significant (Table 4.5). None of the PERMDISP2 analyses were significant.

Lipid content did not differ between sylonized and uninfected shrimp for either whole shrimp or dissected tissues (Table 4.6).

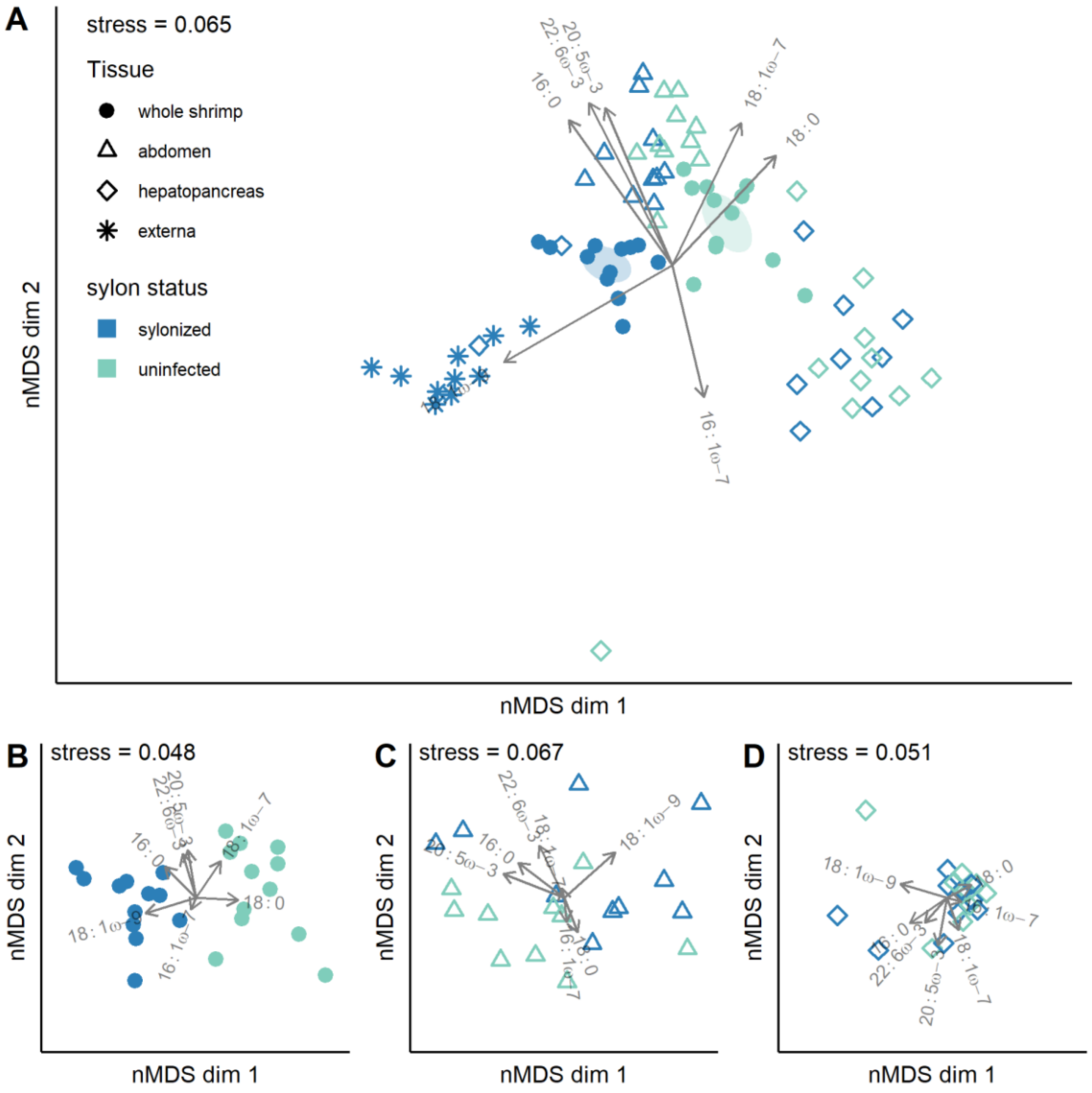

Fig. 4.4. Non-metric multidimensional scaling (NMDS) ordinations of fatty acid (FA) profiles of A) all samples, B) whole shrimp, C) dissected abdomens, and D) dissected hepatopancreases. Ellipses in A show standard errors around the centroids of whole uninfected and sylonized shrimp. Vectors show relative correlations of FA composing at least 5% of the total identified FA of any *Sylon*-tissue combination with NMDS dimensions.

Table 4.5. Permutational multivariate analysis of variance (PERMANOVA) comparisons of shrimp FA profiles. Tissues belonging to the same shrimp were accounted for by using shrimp identity as a stratum for dissected shrimp. In comparisons within tissues (abdomen only and hepatopancreas only), stratum was not used as each sample belonged to a single shrimp. Externae were excluded. PERMANOVAs used Euclidean distances and 9999 permutations.

Analysis	**Variable**	**Df**	**MS**	**Pseudo-F**	**p**
Whole shrimp	*Sylon*	1	232.370	23.863	0.0001
	residuals	22	9.737		
Dissected shrimp	*Sylon*	1	57.46	1.733	0.0001
	Tissue	1	1359.75	41.010	0.0001
	Sylon:Tissue	1	23.58	0.711	0.5261
	residuals	36	33.16		
Dissected: abdomen only	*Sylon*	1	25.415	2.983	0.0426
	residuals	18	8.521		
Dissected: hepatopancreas only	*Sylon*	1	55.628	0.963	0.4205
	residuals	18	57.792		

Table 4.6. Wilcoxon rank-sum tests comparing lipid content of sylonized and uninfected shrimp by tissue type.

Tissue	**W**	**P**
Whole shrimp	71	0.98
Abdomen	59	0.53
Hepatopancreas	50	1.00

3.4. QFASA Diet analysis including sylonized shrimp

QFASA round 1, which used sylonized and uninfected shrimp as separate prey, predicted prey proportions in rockfish diets in descending order: herring (0.37 ± 0.07, mean ± standard error, Fig. 4.5A), sylonized shrimp (0.23 ±0.09), pygmy rock crab (0.13 ± 0.04), sand lance (0.13 ± 0.07), uninfected shrimp (0.08 ± 0.08), and Dungeness crab (0.07 ± 0.03). QFASA round 2, which combined sylonized and uninfected shrimp as a single prey type, predicted similar prey proportions in descending order: herring (0.38 ± 0.07, Fig. 4.5B), shrimp (0.27 ± 0.09), pygmy rock crab (0.15 ± 0.04), sand lance (0.13 ± 0.06), and Dungeness crab (0.07 ± 0.03). In QFASA round 3, predictions were similar to the other two rounds, but increasing the proportion of sylonized shrimp used in the prey

data increased predicted dietary proportions of sand lance and Dungeness crab and decreased the proportions of herring and pygmy rock crabs (Fig. 4.5C). Predicted dietary proportions of shrimp peaked at intermediate levels of proportion sylonized (Fig. 4.5C).

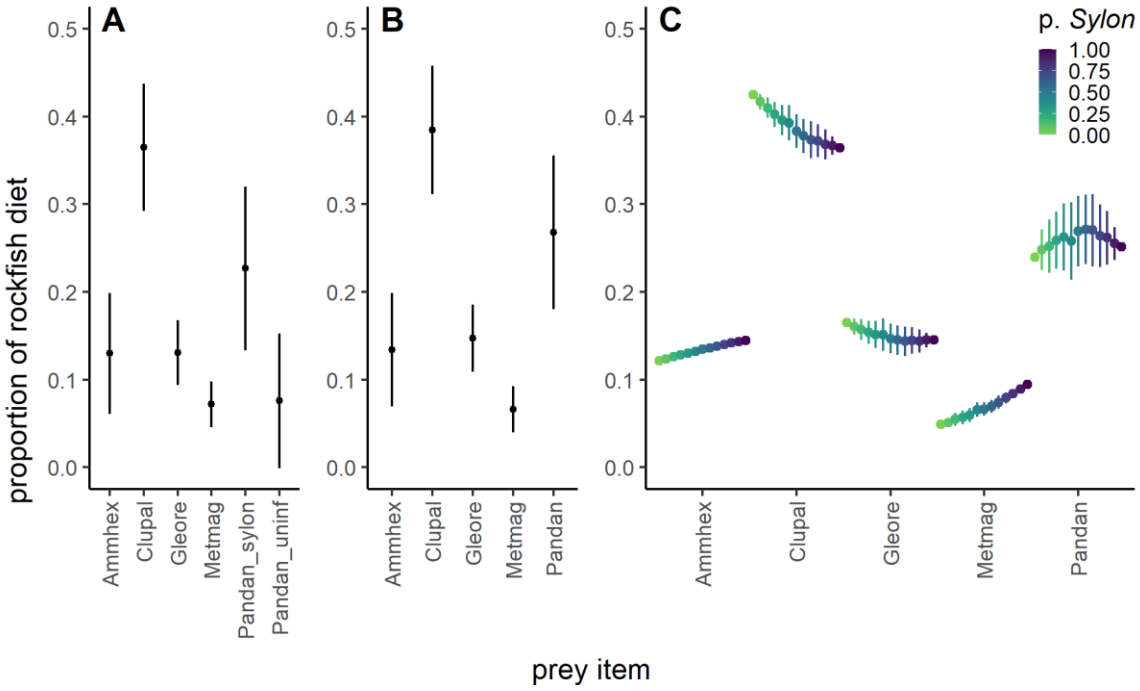

Fig. 4.5. Copper rockfish diet predictions from Quantitative Fatty Acid Signature Analysis (QFASA) models, in A) round 1, where sylonized and uninfected shrimp were separate prey types, B) round 2, where sylonized and uninfected shrimp are combined as a single prey type, and C) round 3, where the proportion of shrimp sylonized (p. *Sylon*) was manipulated through subsampling. Prey are Pacific sand lance (Ammhex), Pacific herring (Clupal), pygmy rock crab (Gleore), Dungeness crab (Metmag), and shrimp (Pandan, _sylon = sylonized, _uninf = uninfected). Error bars in A and B are bootstrapped standard errors of 100 iterations each. Error bars in C are standard deviations of predictions from the 100 subsampled sets of manipulated proportions of sylonized shrimp.

4. DISCUSSION

Sylonized shrimp in our study were found to differ extensively from uninfected shrimp. Sylonized shrimp had greater rates of epibiosis, an onset of female sex characters at a smaller size, and altered FA relative to uninfected shrimp. Altogether, these changes

to shrimp driven by *Sylon* suggest a substantial impact by the parasite and potentially shifted ecological roles.

Higher rates of epibiosis with *Sylon* stage is aligned with the expectation that parasitism's effects would increase with the extent of infection. Duration is likely an important factor, as the longer a parasite interferes with antifouling defenses, the more epibionts are likely to accumulate. A common effect of rhizocephalans is cessation of molting or the increase in intermolt period, though this is not consistent among different rhizocephalan taxa (Høeg 1995). Molting would otherwise clear epibionts from the host surface, and the interference of molting is considered a key connection between rhizocephalan infection and epibiosis in various crabs species (Gaddes & Sumpton 2004, Mouritsen & Jensen 2006, Innocenti & Galil 2011b). Altered molting is thus a likely cause for the patterns observed with *Sylon* and *P. danae*. It should be noted, though, that the transition between a primordial and emerged externa requires a molt (Lützen 1981). This suggests that the intermolt period during which an emerged externa is present must be long enough to reaccumulate epibionts that were lost during the transitionary molt.

A prominent host change associated with rhizocephalan parasitism is feminization of male hosts (Høeg 1995). In most cases, feminization has been studied in hosts that exhibit gonochoric sexual development. *P. danae,* on the other hand, is a protandrous hermaphrodite, starting life as a juvenile, developing into a male and then transitioning to a female. Using size as a proxy for age, we found that sylonized *P. danae* develop female sexual characters earlier than uninfected shrimp. More thorough investigations into feminization effects should include internal observations of shrimp reproductive structures and measurements over time. Indeed, histological investigations have shown

more specific disruptions in shrimp gonads in sylonized *P. platyceros* and *Spirontocaris liljeborgii* (Lützen 1981, Bower & Boutillier 1990a). It is unclear whether this feminization effect is likely to produce substantial reproductive impacts at the population level, especially as infected shrimp regardless of sex are sterilized at least for that reproductive season.

By sterilizing the host, *P. danae* population reproductive rates are likely affected by *Sylon*, as these effects are recognized for other rhizocephalans (Kuris & Lafferty 1992, Torchin et al. 2001). An interesting facet of reallocating host reproductive investments to parasite ones is potential effects to planktonic food webs. *P. danae* produces planktotrophic (feeding) larvae that can function as both predator and prey in planktonic food webs (Bergström 2000, Litz 2017). In contrast, the cyprid larvae of *Sylon* are lecithotrophic (non-feeding), remarkably small for barnacle cyprids (Lützen 1981, Høeg 1995), and likely would not serve the same ecological role as the shrimp larvae they replaced, especially with a short planktonic duration.

Because sterilization represents a substantial reallocation of host resources, we expected lipid measures to differ by sylonization. For example, crabs infected by *Sacculina* had consistently lipid-rich hepatopancreases, indicating parasite-driven changes to lipid metabolism (Smith 1911). Interestingly, lipid content did not differ among whole shrimp nor dissected tissues of *P. danae* depending on *Sylon* infection. We originally expected that lipids in the hepatopancreas could have been diverted to *Sylon* externa in sylonized shrimp, but there was no corresponding difference in lipid content and FA profiles showed considerable overlap in NMDS. This finding is consistent with Smith's (1911) finding of lipid rich hepantopancreases in infected crabs. *Metopograpsus*

thukuhar (Owen) crabs infected by *Polyascus planus* (Boschma) had greater triacylglycerol concentrations in the hepatopancreas relative to uninfected ones but the opposite pattern for hemolymph. With differences in glycogen and glucose allocation in infected and uninfected crabs, these patterns were interpreted as a reallocation of muscle resources to the hepatopancreas (Hsiao et al. 2016). It is likely, then, that lipids or resources from other tissues, such as developing gonads, were diverted to the hepatopancreas and externa with a net neutral effect on shrimp lipid content. Still, FA profiles of whole shrimp differed between sylonized and uninfected shrimp. The difference seems to be greatly driven by greater proportions of oleic acid (18:1ω-9) in sylonized shrimp relative to the uninfected ones. The externa was especially rich in oleic acid, and whole sylonized shrimp had nearly 5% TFA more oleic acid than uninfected ones. Oleic acid, which is a common monounsaturated FA, was the third most abundant FA in sylonized shrimp. In contrast, DHA, a very nutritious, highly unsaturated fatty acid, was the third most abundant FA in uninfected shrimp, though sylonized shrimp still had similar DHA amounts. The implications for the altered shrimp FA for predator nutrition are thus still unclear.

Our QFASA results revealed some notable aspects of sylonization for shrimp FA that may be applicable to other parasite FA studies in the contexts of food webs. Despite whole sylonized and uninfected shrimp being distinct in their FA, they had signatures more similar to each other than to other potential rockfish prey. This was shown in QFASA rounds 1 and 2. In round 1, predictions for separated sylonized and uninfected shrimp showed high variation, suggesting the mixing model was less able to distinguish the two (Fig. 4.5A). In round 2, the combined shrimp still had high variation and was

about the sum of the separated shrimp in round 1, indicating the model could treat them similarly (Fig. 4.5B). However, in round 3, manipulating the proportion of sylonized shrimp used in the shrimp data also directionally changed predictions of sand lance, herring, and Dungeness crab. Predictions for pygmy rock crab generally decreased with proportion of shrimp sylonized, but increased slightly at the highest sylonization proportions. In contrast, the prediction for shrimp was hump-shaped and extreme values of sylonization showed similar predictions (Fig. 4.5C). It might be expected that shrimp predictions would be the most affected by sylonization relative to other prey, as the shrimp FA were those ultimately affected by parasitism. Surprisingly, though, the non-directionality of the shrimp predictions and their large uncertainties suggest that parasite effects in dietary mixing model approaches may not be most reflected in their host. Altogether, our QFASA results show that parasitism can affect host FA in a way that alters mixing model predictions.

The extensive differences we found between sylonized and uninfected shrimp have probable implications for predators of *P. danae* as TMIIs. While we have not established density-related effects of *Sylon* on shrimp (e.g. sterilization outcomes), the changes in epibiosis and fatty acids we found in shrimp might affect the susceptibility of a sylonized shrimp as prey or its nutrition when it is consumed. Epibiosis can both increase and decrease predation rates, depending on the epibiont and the basibiont or host (Wahl & Hay 1995, Wahl et al. 1997, Laudien & Wahl 1999, 2004). The shrimp epibionts, which look qualitatively similar to fouling organisms found on nearby substrate (Yoshioka, pers. obs.), may make shrimp more cryptic, camouflaging them from predators. However, epibionts can increase predation by interfering with sensory

organs and increase hydrodynamic drag (Wahl 1996, Fernandez-Leborans 2010); shrimp observed in our study were sometimes severely fouled on their eyes and antennae, and this species relies on a swimming, tail-flip escape response from predators (Daniel & Meyhöfer 1989, Nemeth 1997). Future research should verify *Sylon*'s potential influence on predation rates. For example, Gehman & Byers (2017) found rhizocephalan-infected crabs were more susceptible to predation in both mesocosm and tethering experiments, and the efficacy of tethering studies has already been demonstrated for *Pandalus* shrimp and their predators (Frid et al. 2012, Yates et al. 2020).

We found that *Sylon* produces multiple and diverse effects on its shrimp host. Despite relatively little research done on rhizocephalan impacts on shrimp, we observed comparable effects in this system relative to crab-rhizocephalan ones including epibiosis and feminization. Still, these similar impacts of *Sylon* on *P. danae* may have especially remarkable and distinct implications compared to carcinized crustaceans, considering the shrimp as a protandrous hermaphrodite and a natant organism. Understanding *Sylon*'s influence in a naturally transitioning host may provide insight into decapod sex determination as well as feminization induced by other rhizocephalans. The influence of epibiosis on hydrodynamics will inform our understanding of energetics and predation risk, which are especially important as pandalid shrimp are valuable fisheries and important prey items. Lastly, we found that FA profiles of sylonized shrimp differed significantly from those of uninfected shrimp, with such FA changes influencing mixing model predictions of predator diets. This study lays the groundwork for future research on a rich rhizocephalan system and underscores the importance of recognizing parasite roles in trophic ecology.

CHAPTER V

CONCLUSION

This work highlights clear examples and potential for trait-mediated indirect interactions of parasites with substantial implications for their ecosystems. Lz and *Sylon* can undoubtedly exert density-mediated indirect effects on their ecosystems. *Labyrinthula* so severely impacted eelgrass beds to cause the first historical extinction of a marine invertebrate in the 1930s (Carlton et al. 1991). Through sterilization, rhizocephalans can limit the populations of their host, most noticeably when their hosts invade new environments (Kuris & Lafferty 1992, Torchin et al. 2001). In the particular situations studied here, DMII were not immediately apparent; the sampled eelgrass bed was qualitatively healthy and the studied *P. danae* were seemingly abundant (Yoshioka pers. obs.). As this work shows, parasites should be considered even when they may most likely be ignored.

Together, Chapters II and III demonstrate Lz's potential contribution to secondary productivity in eelgrass systems, first in producing the nutritionally vital fatty acid DHA and second in facilitating the availability of eelgrass biomass for detritivores. Neither effect requires the death of the eelgrass plant but still may bolster nutrition to eelgrass's consumers and their predators. This clearly constitutes a TMII, as the eelgrass plant, in its form and nutrition, are altered through disease for its consumers (Fig. 5.1A). Through the indirect effect on detritivorous copepods, Lz may also indirectly support their consumers (Fig. 5.1A). The EWD-driven changes of copepod FA are likely less important than EWD's numerical effects on copepod populations, as DHA and EPA, two particularly important FA, did not differ in copepods between study treatments. Future work should

verify Chapter III's effects *in situ* and target the potential for Lz to serve as a nutritious food source for detritivorous copepods. Repeated, in-field surveys at multiple sites relating copepod abundance and EWD prevalence or severity could confirm Lz's realized ecosystem impact. Experiments in which *Tisbe* or other copepods are reared on a pure Lz diet in comparison to other diets will directly address the question of Lz's nutrition.

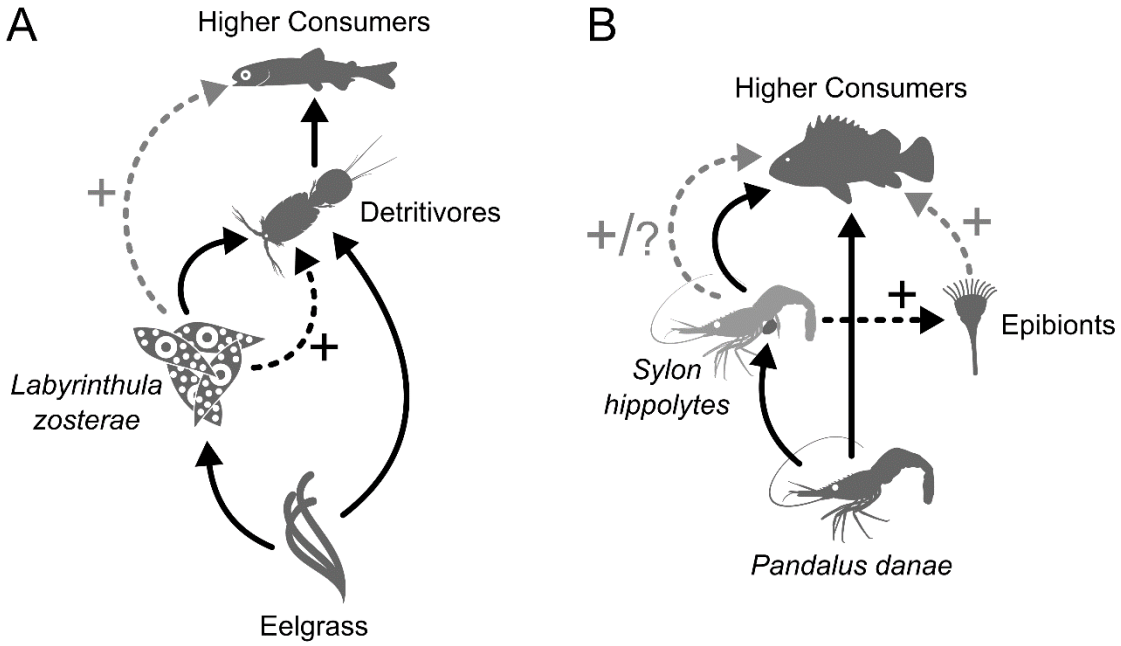

Fig. 5.1. Conceptual diagram of parasite effects in A) the eelgrass wasting disease system and B) the *Pandalus*-*Sylon* system. Solid black arrows show trophic interactions leading toward the consumer. Dashed arrows show indirect effects of parasites or symbionts, with black arrows indicating observed effects and grey arrows indicating hypothesized effects.

In Chapter IV, shrimp infected with *Sylon* exhibited several differences from uninfected conspecifics, indicating pervasive host modification by *Sylon*. First, a greater probability of epibiosis was associated with *Sylon* infection, increasing with *Sylon*'s developmental stage, which is an indirect effect of the parasite on other symbionts of *P. danae* (Fig. 5.1B). Although the mechanism for the association is uncertain, an increase in epibionts with *Sylon* may also make *P. danae* more susceptible to predation. If this is

the case, *Sylon*'s effects on fish predators would be indirectly mediated by two community members, its shrimp host and the epibionts of the shrimp (Fig. 5.1B). Second, *Sylon* altered the FA of *P. danae*, with consequences for dietary analyses of their predators but unclear outcomes for predator nutrition. Third, *Sylon* sterilizes and feminizes its host, with a potential dampening of reproduction. Reproductive impacts and cascading ecosystem effects, if verified, would constitute a DMII. The work presented establishes clear effects of *Sylon* on *P. danae* biology, exposing a study system distinct from the more traditional crab-rhizocephalan systems and one rich for further exploration.

In both parasite systems, hosts are abundant with integral roles for their ecosystems, with eelgrass as a foundation species and *P. danae* as an important prey. In such situations, parasite effects on hosts would be most expected to have substantial community- or ecosystem-level effects (Preston et al. 2016). Just as particular free-living organisms attract research because of the gravity of their ecological effects or their biological relevance, particular parasites do and should be prioritized for study. In other words, not every parasite would be expected to have dramatic ecosystem-level effects. Accordingly, some of the most well-known examples of ecosystem effects of parasites and disease are those affecting abundant or ecologically pivotal hosts (Preston et al. 2016), such as crickets infected by nematomorph worms (Sato et al. 2012), urchins killed in a mass mortality (Lessios 1988), and sea stars plagued by wasting disease (Schultz et al. 2016, Burt et al. 2018). While resources available to research should aim at parasites with especially pivotal effects, the findings of this work underscore that ecological effects of parasites remain understudied.

Finally, the research presented has key, practical implications for FA trophic biomarker studies. In Chapter II, I showed that disease clearly changes the FA of eelgrass, such that the characteristic FA of plant material (ALA) is reduced in Lz-infected tissue. In Chapter III, copepods provided eelgrass did not show an appreciable signature of eelgrass in their FA, even in diseased treatments, where eelgrass segments ultimately produced the biomass consumed by copepods. These results indicate that disease-driven FA changes in host tissues can obscure feeding relationships in biomarker studies. Similar effects may also occur for detrital eelgrass, as microbial "aging" of kelp also shifts its FA (Dethier et al. 2014). FA biomarker studies of seagrass food webs should take these transformations into account, especially when seagrasses are expected to be consumed mostly as detritus.

Similarly, parasite-driven FA changes were also observed in *Sylon*-infected shrimp in Chapter IV. The changes were substantial enough to distinguish sylonized from uninfected shrimp, and also influenced QFASA predictions in a hypothetical rockfish diet analysis. Although the shifts driven by parasitism were subtle in QFASA, they demonstrate the importance of incorporating realistic samples in FA biomarker studies. It is common to sample representative or "normal-looking" samples for FA studies; that approach would have led to sampling only uninfected shrimp, resulting in different predictions from QFASA. More so, if sylonized shrimp are more susceptible to predation, as hypothesized, then QFASA results based on uninfected shrimp would be even less reflective of rockfish diets. Hence, researchers should be thoughtful about sample collection, using truly representative samples instead of pristine ones when appropriate for their study systems.

A key motivation of using biomarker approaches in trophic ecology is that food webs are complicated, with organisms relying on diverse resources that may differ over space and time. Biomarkers like FA help provide an integrated picture of an organism's trophic interactions, as a researcher can interpret an organism's FA as a mixture of those obtained from the various prey the organism consumed (Dalsgaard et al. 2003, Iverson 2009, Kelly & Scheibling 2012). Still, there are vital caveats to FA analysis, such as consumer trophic modification (Galloway & Budge 2020), that still need further study, and the vast majority of food webs remain to unresolved (Pringle 2020). With such difficulties assessing food webs of just free-living species, it is perhaps unsurprising that integrating parasites lags behind, not even considering the theoretical complications in incorporating parasites (Sukhdeo 2010). Further, as predators may indirectly alter the trophic ecology of their prey through non-consumptive effects, parasites too may alter the feeding relationships of their hosts (Werner & Peacor 2003, Lafferty et al. 2008). Uncovering these effects and including parasites as non-optional members of food webs can only improve our understanding of food web and ecological dynamics. The work presented here adds to that effort, showing parasite roles in affecting host nutrition and propelling the transfer of biomass. Interrogating the realized impacts of Lz and *Sylon* on their ecosystems requires further, field-based study, and these and many other systems offer rich opportunities for further exploration. The tree of life's branches are adorned with parasites (de Meeûs & Renaud 2002, Larsen et al. 2017); do we really understand food webs without them?

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