What makes *Elysia viridis* tick?

Fitness Consequences of Diet Selection and Kleptoplasty

Ph.D. Thesis
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ABSTRACT

Understanding the mechanisms involved in resource use by an organism is pivotal to understanding its ecology. A conspecific population that as a whole demonstrates a generalist pattern of resource use may in fact consist of relatively specialized individuals. *Elysia viridis*, a sacoglossan opisthobranch mollusc, tends to demonstrate this type of sympatric variation in diet, although to differing extents depending on the ontogenetic stage. However, the mechanisms underlying this inter-individual variation are poorly understood. Utilizing the basic framework of optimal diet theory, this thesis investigated the prevalence of individual specialization and its effects on energy assimilation in *E. viridis* on different algal diets and the mechanisms that underpin or constrain diet selection. This was assessed through a combination of laboratory experiments addressing how *E. viridis*' original algal host affected algal diet choice, handling efficiency, growth, and the retention of functional chloroplasts (kleptoplasty) in the lab and relating conclusions from these experiments to observations of abundance and size of the sea slug in the study area.

Assessments of abundance and size distributions of *E. viridis* on different algal hosts demonstrated that the sea slug commonly colonized the co-occuring algal species *Codium fragile*, *Cladophora rupestris*, and *Cladophora sericea* in the field. Abundance was generally highest on *Cladophora* hosts compared to *C. fragile* hosts, and *C. rupestris* tended to accommodate larger individuals compared to the other hosts (**paper I**). In the lab *E. viridis* tended to select algal diets that had a similar morphology (filamentous septate vs. planar siphonaceous) to their original host, which related to increased handling efficiency through previous experience of feeding techniques required for different algal morphologies. This indicated that short-term diet selection was influenced by differences in feeding efficiency, suggesting *E. viridis* were specialised to feed on particular diets. However, diet selection did not correlate to the long-term fitness value of a diet, indicating that factors other than nutrition are important for host/diet selection in *E. viridis*. However, positive growth by *E. viridis* on all algal diets irrespective of their original algal host indicated that slugs were capable of effectively switching to non-host algae (**paper II**). Furthermore, *E. viridis* derived functional kleptoplasts from three different genera of algae (*Chaetomorpha*, *Codium*, and *Cladophora*), refuting claims that members of Cladophorales were unsuitable sources of functional kleptoplasts to *E. viridis* (**paper III**). However, kleptoplast functionality varied within the genus *Cladophora*. Finally we provided evidence that *E. viridis* receives a substantial fitness benefit under satiation by retaining functional kleptoplasts through increased growth efficiency via phototrophy (**paper IV**).

Overall this thesis contributes substantially to understanding the fitness trade-offs *E. viridis* faces through diet selection. Furthermore, it emphasizes that assessing energy assimilation in polyphagous sacoglossans requires not only an understanding of the fitness contributions of nutritional and morphological traits of different algal diets but also their role as a source of functional kleptoplasts to the slug. However, many conclusions reached in the lab did not concur with field patterns, indicating a need for further study on biotic (e.g. predation) and abiotic (e.g. wave force) pressures on *E. viridis* populations and assessment of the roles algal hosts play in circumventing these pressures.

Keywords: *Elysia viridis*, sacoglossan, herbivore, macroalgae, seaweed, optimal diet theory, individual specialisation, diet selection, kleptoplasty, phototrophy

LIST OF PAPERS

This thesis is based on the following papers:

Paper I:	Baumgartner FA, Toth GB (manuscript) *Elysia viridis* abundance and size distribution on co-occurring algal hosts on the Swedish west coast.
Paper II:	Baumgartner FA, Pavia H, Toth GB (manuscript) Individual specialization to non-optimal hosts in a polyphagous marine invertebrate herbivore.
Paper III:	Baumgartner FA, Toth GB (manuscript) Kleptoplast functionality in a sea slug varies depending on its macroalgal diet.
Paper IV:	Baumgartner FA, Pavia H, Toth GB (manuscript) Acquired phototrophy through retention of functional chloroplasts increases growth efficiency of the sea slug *Elysia viridis*.

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INTRODUCTION

Unravelling the mechanisms that underlie an animal's selection of an ecological niche is a complex problem that requires a multipronged approach. In general, the first 'port of call' in understanding these mechanisms is an animal's diet (Sih & Christensen 2001). Information on how an organism searches for food, selects between different food types, and chooses to leave a particular food source in search of another, provides basic information towards answering the question of why they fill a particular role in a habitat. Locating and exploiting a food source can be a major source of energy expenditure for a consumer. Hence, it is supposed that a forager will select the most cost efficient method to locate and consume food items that maximize their ability to grow and reproduce. Analysing this cost to benefit has traditionally be conducted through the framework of optimal foraging models collectively known as Optimal Foraging Theory (OFT) (Emlen 1966, Macarthur & Pianka 1966, Schoener 1971, Pyke et al. 1977, Hughes 1980, Pyke 1984, Stephens & Krebs 1986), which attempts to predict why a forager chooses to exploit only some of the many food sources that are potentially available (Svanbäck & Bolnick 2005).

In its simplest form, OFT can be described as addressing decisions of where a forager should search for food, what it should eat, when it should eat and when it should leave to find something else (Owen-Smith et al. 2010). OFT revolves around one basic premise, energy maximisation (Hughes 1980). The Energy Maximisation Premise assumes that a forager will select a foraging strategy that maximises a particular trait related to fitness (e.g. growth, reproduction, survival etc.), which confers a selective advantage. OFT is applicable to many types of foragers, albeit with adaptation of models to suit different foragers types, and therefore both carnivores and herbivores are considered as predators differentiated only by the trophic level of their prey (Hughes 1980). Initial developments of the theory were designed to address inter-specific variation in diet selection (e.g. Schoener 1971), however, OFT has subsequently been extended to address intraspecific/inter-individual resource use (e.g. Svanbäck & Bolnick 2005). Pyke (1984) partitions the theory up into five categories that assess various aspects of foraging behaviour: optimal patch choice, optimal patch departure rules, optimal movement, optimal central place foraging and optimal diet. It seems unnecessary to provide an overview of these categories, as not all are relevant to this thesis. Instead I focus on optimal diet theory (ODT) in relation to variation in diet selection amongst sympatric conspecifics.

Optimal Diet Theory

Sympatric conspecifics that, as a population, exhibit a generalist pattern of resource use may in fact be composed of relatively specialised individuals (reviewed in Bolnick et al. 2003). Previously, many ecological studies have chosen to ignore sympatric conspecific variation in resource use, however it is not uncommon in natural systems (reviewed in Bolnick et al. 2003, Araújo et al. 2011). Optimal diet models provide a framework to understand why a group of conspecific individuals with access to the same set of resources may choose to differentially exploit them (Svanbäck & Bolnick 2005). They address instances where an individual must select between food items from a range of available resources, for example selecting between food types within a patch (Hughes 1980). Models assume that an individual will select a diet that maximizes some currency related to fitness (e.g. growth, reproduction etc.). Individuals are assumed to be able to rank food items in terms of their fitness value and are predicted to select the highest ranked food available. In general the value of a particular diet is measured as a rate of energy assimilation per unit foraging time (Hughes 1980). This rate may be dependant on a number of factors such as the energetic content of alternative food types, rates of encounter with particular food types, times spent searching (search times), recognizing (recognition times) and handling food types (handling times), and experience of food types (Pulliam 1974, Werner & Hall 1974, Hughes 1979, Hughes 1980). If sympatric conspecifics differ in their ability to locate, recognize, and handle different food types this may result in individual specialisation, whereby individuals within a population differentially exploit available resources within a patch or habitat (Svanbäck & Bolnick 2005).

Rates of encounter are generally assumed by assessing the relative abundances of different food types in a habitat or patch to provide the likelihood that an individual will encounter a particular food type (Schoener 1971, Lubchenco & Gaines 1981). Search time is the time spent searching for a food type exclusive of recognizing and handling it. Recognition time relates to the time taken for an individual to recognise and evaluate a food type when it is encountered. Initial diet models omitted this factor as they were designed for visual predators and assumed this cost to be negligible. However, the concept was introduced into subsequent diet models (e.g. Hughes 1979) as it was considered a potential search cost relevant to foragers that use potentially slower means of food detection such as chemoreception or touch (Hughes 1980). Handling times refer to the time taken by an individual to consume a food item after search, encounter, and recognition (Hughes 1980). This could be dependent on a number of factors such as the size (e.g. Palmer 1983) and nutritional content of the food (e.g. Barile et al. 2004) and the ease of its ingestion and digestibility with relation to mechanical or chemical defences (e.g. Pennings & Paul 1992).

Experience or learned behaviour is conceivably an important determinant of an individual's diet. A particular food type may change in rank if experience decreases handling times resulting in increased energy assimilation per unit handling time. Furthermore, experience of a particular food type may lead to development of visual or chemical search images that drastically reduce search and recognition times thereby increasing effective encounter rates (Lewis 1986, Daniel & Bayer 1987). If this were to occur, an individual would be assumed to become more efficient at selecting a certain food type whilst ignoring others, resulting in reduced costs involved in search for and recognition of suboptimal foods.

Deviations from Optimal Diet Theory

Optimal diet models have received criticism (e.g. Pierce & Ollason 1987), although a review (Sih & Christensen 2001) provides support for their use with foragers that have immobile prey, such as herbivores. However, it would be naïve to think that the energy assimilation per unit foraging alone would explain all aspects of individual diet selection. In natural systems many other pressures such as intraspecific competition (e.g. Holbrook & Schmitt 1992), sex (e.g. Jormalainen et al. 2001), removal by waves (e.g. Sotka 2007), predation etc. are likely to influence or constrain an individual's diet selection. These factors could be just as important, if not more so, in deciding an individual's diet. For example, predation can have a strong impact on habitat use and foraging behaviour in a variety of animals, causing a forager to select diets or hosts predicted to be sub-optimal to either stave off or avoid predators (e.g. Stachowicz & Hay 1999, Williamson et al. 2004). Furthermore, for small herbivores (mesograzers) that reside on the plants they consume, food and habitat are closely tied so the refuge value of a host may be as important as its dietary value, or more so, in determining patterns of diet selection (Duffy & Hay 1991, Jormalainen et al. 2001, Warfe & Barmuta 2004). Hence, as is aptly pointed out by Hughes (1980) and Stephens & Krebs (1986), simple optimal diet models should serve as a starting point for assessing various aspects of diet selection. If predictions of the models explain a large proportion of diet selection under controlled conditions, it might then be easier to identify factors that cause deviations in natural systems. With this in mind I now introduce my study organism *Elysia viridis* with a brief description of the order sacoglossa and the state of knowledge on *E. viridis* ecology with respect to resource use.

Order Sacoglossa

Sacoglossans, or sea slugs, are a diverse order of molluscs in the subclass Opisthobranchia that are thought to be one of the few examples of marine specialist herbivores (Hay 1992, Hay & Steinberg 1992, Williams & Walker 1999, Krug 2011 and references therein). One of the defining characteristics of the order is the possession of a highly modified uniseriate radula that is used to pierce and suck cytoplasm from their foods, of which the majority are green siphonaceous macroalgae (Jensen 1983, Clark 1992). In addition to a specialised feeding apparatus, sacoglossans have a number of other functional specialisations with their food including crypsis in both morphology (Marín & Ros 2004) and colouration (Clark & Busacca 1978), the sequestration or synthesis of natural products for defence (Paul & Van Alstyne 1988, Hay et al. 1990, Gavagnin et al. 1994, Becerro et al. 2001, Marín & Ros 2004), as well as the sequestration of functional chloroplasts (kleptoplasty, *sensu* Raven et al. 2009) as a supplementary energy source through photosynthesis (e.g. Hinde & Smith 1975, Clark et al. 1990, Green et al. 2000, Evertsen et al. 2007). Kleptoplasty has been well studied in recent years however efforts have focussed more attention on the length of time that kleptoplasts remain functional under conditions of starvation (Hinde & Smith 1975, Pierce et al. 1996, Evertsen et al. 2007, Evertsen & Johnsen 2009, Yamamoto et al. 2012) with the only benefits of kleptoplasty being suggested for times of food shortage (Hinde & Smith 1975, Evertsen et al. 2007, Casalduero & Muniain 2008). Relative contributions of heterotrophy through grazing and phototrophy through kleptoplasts to sacoglossan energy budgets are largely unknown (Clark 1992, Williams & Walker 1999, Teugels et al. 2008, but see Marín & Ros 1992).

Sacoglossan body morphology is variable and the order is generally divided into shelled (complete or reduced) and unshelled suborders (Williams & Walker 1999). In general terms most species tend to be restricted to one genus of seaweeds. However, shelled species are thought to have narrower dietary ranges than unshelled species with inter-generic dietary ranges more common in the unshelled genus *Elysia* (Jensen 1997, Curtis et al. 2006, Händeler & Wägele 2007, Middlebrooks 2012). Despite sacoglossans being considered to have specialized diets, there is at least one species, *Placida dendritica*, which has well documented evidence of inter-generic diet specialisation in sympatric conspecifics (Trowbridge 1991, Bolnick et al. 2003). *P. dendritica* demonstrates an extreme limitation to host switching and generally dies in the presence of non-preferred diets that other conspecifics consume (Trowbridge 1991). *E. viridis* demonstrates similar diet specialisation in sympatric individuals however there appears to be more flexibility in diet switching to non-preferred species (Jensen 1989).

***Elysia viridis*: a candidate for understanding sympatric conspecific variation in resource use**

E. viridis is an unshelled sacoglossan that occurs throughout the Northeast Atlantic from Norway through the British Isles, to the Mediterranean and North Africa (Trowbridge 2000 and references therein). It is one of the few sacoglossan species that has been relatively well studied in terms of its ecology (Jensen 1989, Jensen 1993, Trowbridge & Todd 2001, Trowbridge 2002, Trowbridge 2004, Trowbridge et al. 2008), reproductive biology (Trowbridge 2000, Trowbridge & Todd 2001), and kleptoplasty (Trench 1975, Hinde & Smith 1975, Evertsen & Johnsen 2009, Viera et al. 2009). Furthermore, it is one of the few sacoglossan species that consumes algae from a number of different taxonomic groups including the siphonaceous/coenocytic green algal genera *Codium* and *Bryopsis*, the filamentous/septate green algal genera *Chaetomorpha* and *Cladophora*, and the filamentous red algal species *Griffithisia devoniensis* (Jensen 1989, Trowbridge 2000, Trowbridge et al. 2008).

The basic consensus from diet studies is that previous host history/experience is important in *E. viridis* diet selection, particularly in adults, with positive feeding preference induction generally occurring (Jensen 1989, Trowbridge & Todd 2001). However, this trend is not as prevalent in postlarvae (Trowbridge & Todd 2001). Diet switching is possible to some extent (Jensen 1989, Trowbridge & Todd 2001), although it appears limited by the ability to learn different feeding techniques required for morphologically variable diets (e.g. filamentous vs. planar algae, Jensen 1989). Switching may be related to differences in tooth morphology, which are thought to be inducible by diet (Jensen 1993). However, recent comparisons of sympatric *E. viridis* have suggested tooth shapes of individuals from different algal hosts, both septate and siphonaceous, are relatively similar (Trowbridge et al. 2008). So it may be that experience of a particular feeding technique may be of more relevance than tooth shape in deciding diet selection.

Although there appears to be evidence of inter-individual specialisation in *E. viridis* populations the mechanisms driving this specialisation and the trade-offs involved with specialising have not been sufficiently elucidated. For example, there has been no attempt to assess how *E. viridis* growth correlates with diet nutrition or morphology. Moreover, studies of growth using individuals with different host histories have not been conducted in parallel, making it difficult to compare the impact of host history on energy assimilation. Diet selection experiments have relied on host associational choice assays with no considerable attempts to understand the relative contributions of handling efficiency or morphology in diet selection (but see Jensen 1989). One striking omission has been the benefits of kleptoplasty to *E. viridis* fitness in terms of increased growth efficiency and the extent to which it occurs on species other than *Codium*. Although kleptoplasts derived from *Codium fragile* have a demonstrated fitness advantage under starvation attempts to assess fitness under satiation ultimately failed (Hinde & Smith 1975). Furthermore, *E. viridis* are assumed to be unable to derive functional kleptoplasts from algal genera other than *Codium* (Trowbridge & Todd 2001), however this has never been explicitly tested.

In the archipelago around Tjärnö, *E. viridis* occur sympatrically on several genera of macroalgae with varying morphologies i.e. septate and siphonaceous cell structure, simple and complex gross morphology. Therefore, it is an appealing system to assess within population variation in diet selection, growth, and kleptoplasty with relation to host history/experience.

Aims of the thesis

This PhD work aimed to improve the understanding of the mechanisms underlying *E. viridis* diet selection by addressing questions that had, in my opinion, been poorly assessed or overlooked in areas of *E. viridis* diet selection. Following the example of Jensen (1989), I used the framework of optimal diet theory to qualitatively test aspects of *E. virdis* diet selection, predominantly the effect of host history/experience. This amounted to assessing the effect of *E. viridis*' previous diet history on diet choice, handling time, kleptoplast functionality, and how this related to energy assimilation in *E. viridis* individuals fed different algal diets. This thesis contains four separate papers and the following section describes their specific aims:

Paper I: Observational field data are important in providing an ecological basis to test and/or explain how mechanistic studies fit within the contextual framework of natural systems (Underwood et al. 2000). Previous reports of *Elysia viridis* on the west coast of Sweden (Thomasson & Tunberg 2005, Nohrén et al. 2009) provided no information on size, abundance or host associations. Hence, to provide a framework with which to relate subsequent mechanistic studies in the lab we assessed the seasonal patterns of size and abundance of sympatric *E. viridis* and establish if they commonly exploited co-occurring intergeneric and congeneric algal hosts.

Paper II: From **paper I** it was clear that sympatric *E. viridis* consistently associated with three co-occuring algal hosts of both filamentous septate (*Cladophora rupestris* and *Cladophora sericea*) and planar siphaceous (*Codium fragile*) morphologies in the field and that slugs were in greater abundance on *Cladophora* spp. than *Codium fragile*. Using *E. viridis* collected from these three algal host species we compared the short-term diet choice, consumption rate (handling efficiency), and long-term growth (a proxy for fitness), of slugs on four different algal diets (three algal hosts plus *Chaetomorpha melagonium*) in the laboratory. Furthermore, we measured several nutritional and morphological algal traits in order to determine if they correlated with the fitness value of the different diet species. We specifically hypothesized that if *E. viridis* individuals are specialized to live on, and feed from, different algal hosts, slugs will associate with their original algal host species when given a choice, and have a higher consumption rate on their original algal host species in the laboratory. Furthermore, if intraspecific competition affected individual specialization, we hypothesized that *E. viridis* populations collected from *Cladophora* hosts would have a higher degree of individual specialization compared to *E. viridis* from *C. fragile*. Moreover, if *E. viridis* individuals were able to rank and choose food items in terms of their fitness value as predicted by the ODT, slugs collected from different algal hosts in natural field populations would show the highest growth rate on the original algal host species.

Paper III: Results from **paper II** assessed the effect of *E. viridis* algal host on diet choice, handling efficiency, and growth related to algal diet nutrition and morphology. However, the study did not assess the potential roles of kleptoplasty. Previous assertions were that *E. viridis* were incapable of retention of functional kleptoplasts from algal genera other than *Codium*, specifically that diets from Cladophorales (including *Chaetomorpha* and *Cladophora*) were unsuitable donors of functional kleptoplasts to *E. viridis* (Clark et al. 1990, Trowbridge & Todd 2001). However, these assertions had never been explicitly tested. Using pulse amplitude modulated (PAM) fluorometry, we assessed the variation in functional kleptoplasty of *E. viridis* from different algal hosts and switched to non-host diets. We specifically hypothesized 1) that kleptoplasts in *E. viridis* originating from different algal hosts would differ in functionality, 2) *E. viridis* from different hosts would take up functional kleptoplasts from non-host diets, but the functionality would differ depending on the original host and diet species, 3) the functionality of retained kleptoplasts would decrease during starvation, and the functionality decrease depending on the original algal host and algal diet species, 4) if slugs gain a fitness advantage by retaining functional kleptoplasts, the mass change of *E. viridis* during starvation would be positively correlated to the functionality of retained kleptoplasts.

Paper IV: Fitness benefits of kleptoplasts through phototrophy to sacoglossans with access to food are poorly understood. Furthermore, results from **paper III** indicated that kleptoplast functionality varied in *E. viridis* dependant on original algal host or algal diet. In particular, *E. viridis* that had kleptoplasts derived from *C. fragile* demonstrated high photosynthetic functionality whereas individuals with *C. rupestris* derived kleptoplasts had limited functionality. Hence, using this natural variation we assessed the possible fitness benefits (increased growth efficiency) of functional kleptoplasts under ecologically relevant light and food conditions. Growth efficiency was measured in *E. viridis* fed two algal host species (*C. fragile* and *C. rupestris*) under two light regimes (high and low) for four weeks. Because *C. fragile* was known to provide highly functional kleptoplasts we hypothesized that *E. viridis* originating from, and maintained on, this diet would show higher growth efficiency and relative electron transport rate (rETR) in high compared to low light. In contrast *E. viridis* originating from and maintained on *C. rupestris* would show a similar growth efficiency and rETR irrespective of the light regime. Furthermore, if *E. viridis* received a fitness benefit due to an increased rETR through functional kleptoplasts, the growth efficiency would be correlated to the rETR, but not to any nutritional or morphological traits, of the macroalgal diets.

METHODS

Study area and field collections

All laboratory experiments were conducted at the Sven Lovén Centre for Marine Sciences-Tjärnö under stable conditions indoors. For laboratory experiments *E. viridis* were collected from three algal species (*Codium fragile* (Suringar) Hariot 1889, *Cladophora rupestris* (Linnaeus) Kützing 1843*,* and *Cladophora sericea* (Hudson), Kützing 1843) that were collected from two sites (Yttre Vattenholmen 58° 52’ 33.5’’ N, 11° 6’ 22.9’’ E and Saltö Lyngnholmen 58° 51’ 45.3’’ N, 11° 7’ 52.8’’ E) in the Koster Fjord, Sweden by snorkelling. As *E. viridis* are difficult to locate directly in the field algal individuals were collected in separate press seal bags filled with seawater and taken back to the lab to be searched for *E. viridis*. Collected individuals were kept in 5 L aquaria (separated by algal host collected from) outdoors and fed *ad libitum* diets of their original host as food for at least a month prior to their use in laboratory experiments (**papers II, III,** and **IV**). The three algal hosts and *Chaetomorpha melagonium* (F. Weber & Mohr) Kützing 1845, were used as algal diets in experiments. All algal material used as diets in experiments were collected from the same sites and kept in 5 or 20 L aquaria for maximum of 10 days prior to their use in experiments.

PAM fluorometry

Measuring chlorophyll *a* fluorescence using PAM fluorometry is powerful method to assess properties of the photosynthetic apparatus. In **papers III** and **IV** we used this method in several capacities to assess certain aspects of the functionality of photosystem II reaction centres (PSII RCs) of chlorophyll *a* of both kleptoplasts in *E. viridis* and chloroplasts of subsamples of algal individuals they were provided as diets. PAM fluorometers employ three or four different light sources to manipulate the photosynthetic apparatus (specifically the chlorophyll *a* molecule), causing emission of different quantities of fluorescence. These include the measuring light (ML), saturating pulse (SP), actinic light (AL), and far-red light (FR). I used a diving-PAM (Walz, Effeltrich, Germany), which does not have a FR.

Firstly, a weak ML (< 0.15 µmol quanta m^{-2} s^{-1}) produces a fluorescence emission from the sample without inducing photosynthesis, and this is used to assess the proportion of closed PSII RCs. Once a PSII reaction centre captures a photon, it must transfer the energy to the electron transport chain before it can re-open (re-oxidize) and capture another photon. Fluorescence emitted as a result of the ML only, is called minimum fluorescence (F_o for dark-adapted and F for light-adapted samples as measured immediately prior to the saturating pulse). The second light source is a short SP (up to 18000 µmol quanta m^{-2} s^{-1}, up to 1 s), which is used to assess photosynthetic activity by temporarily closing (reducing) all PSII RCs, resulting in a substantially greater fluorescence emission, referred to as the maximum fluorescence (F_m if dark-adapted or F_m' if light-adapted). AL generates continuous light (up to 6000 µmol quanta m^{-2} s^{-1}) that is used to induce photosynthesis resulting in simultaneous changes in fluorescence (Ralph & Gademann 2005, Roháček et al. 2008). In our experiments we did not use AL but instead induced photosynthesis using fluorescent lights (**paper IV**). Photosynthetic efficiency can be derived from the minimum and maximum values. Maximum quantum yield of PSII ($F_v/F_m = (F_m - F_o)/F_m$, requires dark-adapted samples) quantifies the maximum photochemical capacity of open PSII RCs and is often used to indicate stress or damage to PSII RCs (Roháček et al. 2008). Effective quantum yield of PSII ($\Delta F/F_m' = (F_m' - F)/F_m'$ requires light-adapted samples) provides an indication of the amount of energy used in photochemistry related to the fraction of photochemically active PSII RCs (Roháček et al. 2008). Effective quantum yield measured with light-adapted samples are lower than dark-adapted samples (F_v/F_m), due to the inherent impact of non-photochemical quenching reducing the light-adapted quantum yield (Beer et al. 1998). Relative electron transport rate (rETR) is often used to approximate the rate that electrons are pumped through the photosynthetic chain, providing an indication of electron transport through photochemical reaction leading to carbon fixation (Ralph et al. 1998, Ralph & Gademann 2005). It is usually described from a relationship between irradiance, leaf absorbance and quantum yield, which often relates to oxygen evolution or CO_2 uptake (e.g. Beer et al. 1998, Genty et al. 1998):

$$\text{rETR} = \Delta F/F_m' \times \text{PPFD} \times \text{AF} \times 0.5$$

PPFD represents the photon flux density (µmol quanta m^{-2} s^{-1}), AF relates to absorption factor of the sample (0.84 for an average leaf), 0.5 compensates for absorbed light energy being split between PSI and PSII (Ralph & Short 2002). However, as we had no knowledge of AF, nor if the assumption that the absorbed light energy was split between the photosystems was justified in our slugs or algae we chose to omit them, simplifying rETR calculations to:

$$\text{rETR} = \Delta F/F_m' \times \text{PPFD}$$

In **paper III** we measured $\Delta F/F_m$’ at 5 μmol quanta m^{-2} s^{-1} in *E. viridis* and algae that had been kept under low light levels (4-8 μmol quanta m^{-2} s^{-1}) to provide a relative estimate of kleptoplast functionality in individuals from different algal hosts and after being switched to non-host algal diets. It may have been more appropriate to have dark-adapted all samples and measured maximum quantum yield (F_v/F_m), as we did in subsequent starvation experiments. However, we assumed the low light levels that *E. viridis* were kept under would not have been heavily affected by irradiance, down-regulation, and non-photochemical quenching (Schreiber et al. 1994). This allowed us to assess a larger number of *E. viridis* and algae in a short period of time and avoided long periods of dark adaptation that would have been required.

In **paper IV** we used F_v/F_m as a check to ensure that the photosynthetic capacity of PSII RCs were equal in each group of *E. viridis* and algal individuals prior to their use in different light treatments. Furthermore, we used $\Delta F/F_m$’ in conjunction with PPFD to assess the consistency of rETR in *E. viridis* and algal diets. This provided and indication of electron transport through photochemical reaction leading to carbon fixation (Ralph et al. 1998). This measure has been shown to correlate well with measures of O_2 generation and CO_2 uptake in seagrasses and higher plants (Beer et al. 1998, Genty et al. 1998). However, correlations between PAM measurements and O_2 generation and CO_2 uptake have not been assessed in *E. viridis*. Although, CO_2 uptake has been shown to increase as a function of light irradiance for *E. viridis* with *C. fragile* derived kleptoplasts (Hinde & Smith 1975), indicating that increases in rETR likely indicate increased photosynthesis.

RESULTS AND DISCUSSION

Paper I demonstrated that sympatric *E. viridis* consistently colonized three species of macroalgae *Cladophora sericea*, *Cladophora rupestris*, and *Codium fragile* in large numbers during the summer and to lesser extent during the autumn. Furthermore, *E. viridis* abundance tended to be higher on *Cladophora sericea* and *Cladophora rupestris* compared to *Codium fragile* and *E. viridis* were larger on *Cladophora rupestris*. This indicated that intraspecific competition was likely to be higher on *Cladophora* hosts. Results both agreed and conflicted with other studies conducted in the British Isles (Trowbridge & Todd 2001, Trowbridge 2004, Trowbridge et al. 2008). **Paper I** provided a solid ecological platform with which to compare and contrast conclusions from laboratory-based studies in the subsequent papers.

Paper II demonstrated *E. viridis*' tendency to select diets of similar morphology to their original algal host (i.e. filamentous septate vs planar siphonaceous) in the lab, which related to their short-term ability to consume algal diets. Diet selection, however, was independent of the fitness value of algal diets, indicating that factors other than nutrition are important for host/diet selection in *E. viridis*. Together this indicated that individuals are specialized to live and feed on their algal host similar to *P. dendritica* (Trowbridge 1991). However, settlement on a particular algal host does not preclude successful switching to non-host diets by *E. viridis* as the ranking of an algal diet with respect to its fitness value was correlated to cell/utricle volume and largely unaffected by an individual's original algal host. Particularly striking was that, regardless of algal host, *E. viridis* grew 5 times larger when fed *Chaetomorpha melagonium*, the only completely non-host algal diet, compared to *Codium fragile* with growth on both *Cladophora* spp. similar but approximately half that of *C. fragile*.

Paper III refuted previous assertions that members of Cladophorales were unsuitable sources of kleptoplasts for *E. viridis*. *E. viridis* retained kleptoplasts that demonstrated moderate to high functionally from three genera of algae (*Codium*, *Chaetomorpha,* and *Cladophora*). However kleptoplast functionality in *E. viridis* varied within the genus *Cladophora* with *C. rupestris* derived kleptoplasts demonstrating little to no functionality compared to *C. sericea*, which displayed moderate functionality. Furthermore, *E. viridis* showed uptake of kleptoplasts from non-host diets indicating that settlement on a particular host did not preclude switching between kleptoplast sources. However, PAM fluorescence measurements of kleptoplast functionality did not correlate well with performance traits (i.e. relative mass loss) under starvation. This indicated that PAM fluorometry needs to be coupled with other measures (e.g. O_2 generation or CO_2 uptake per unit mass or chlorophyll *a* content) to quantitatively assess benefits of kleptoplast functionality under starvation. However, by using PAM-fluorometry and having control of the algal dietary history of *E. viridis* we were able to demonstrate that kleptoplast functionality varies in *E. viridis* dependant on the algal diet species it feeds on.

Paper IV demonstrated that functional kleptoplasts afforded a fitness benefit to *E. viridis* through increased growth efficiency. Growth efficiency almost doubled under high light compared to low light in *E. viridis*, originating from and fed a diet of *C. fragile*, which linked to increased rETR in high light. In contrast growth efficiency did not differ between light treatments for *E. viridis* originating from and fed on *C. rupestris*, and rETR of individuals were low regardless of light treatment. Growth efficiency differences, or lack thereof were attributed to differing functionality of kleptoplasts derived from *C. fragile* (high functionality) compared to *C. rupestris* (low to limited functionality) as nutritional and morphological difference between algal diet species, kept under the same light treatments as *E. viridis*, were small. Together this indicates that kleptoplasts can mediate energy acquisition through phototrophy and feeding on algal diet species that provide kleptoplasts of limited functionality (i.e. *C. rupestris*) may represent a potentially large fitness trade-off to *E. viridis*.

CONCLUSIONS AND FUTURE DIRECTIONS

Overall this thesis highlights that sympatric *E. viridis* consistently colonize several different algal species in the field (**paper I**). *E. viridis* are specialized to different hosts but do not select diets based on energy maximisation (**paper II**) nor kleptoplast functionality (**paper III**). However, *E. viridis* still maintain the flexibility to effectively switch (**paper II**) and derive kleptoplasts from new diets (**paper III**). Furthermore, algal diet selection can have fitness consequences for *E. viridis*, which relate to nutrition gained per unit feeding effort (**paper II**) but also potentially to the functionality of kleptoplasts derived from their diet (**paper IV**).

Future studies should focus on establishing if the kleptoplast functionality in *E. viridis* demonstrated on the other algal diets in **paper III** (e.g. *C. melagonium*, *C. sericea*) also translates to increased growth efficiency, as it did for *C. fragile*, as well as on other known algal diets of *E. viridis* (see Händeler & Wägele 2007). Furthermore, more accurate estimates of the contribution of heterotrophy through grazing and phototrophy through kleptoplasty to the energy budget of *E. viridis* are certainly required along with assessment of their correlation with other fitness measures (e.g. reproduction). Finally, *E. viridis* may provide a good model organism to aid ongoing efforts to determine the mechanisms that allow kleptoplasts to remain functional in sacoglossans (e.g. Rumpho et al. 2008, Wägele et al. 2011, Bhattacharya et al. 2013). The discovery that the functionality of kleptoplasts derived from different algal species by *E. viridis* varies (paper III) should provide a platform to compare and contrast kleptoplast stability and genetic integration within a single sacoglossan host species.

While this thesis has contributed further insight into aspects of energy assimilation in *E. viridis* many of the conclusions from lab experiments did not align with patterns of size and abundance on algal hosts in the field. For example, based on our results it would have been assumed that *C. fragile* would represent a superior host for *E. viridis* compared to *C. rupestris* due to increased growth and growth efficiency from functional kleptoplasts. However, sympatric *E. viridis* were often larger and more abundant on *C. rupestris* than *C. fragile* in the field. This indicates that other factors unrelated to energy maximisation determine host suitability in the field. *E. viridis*, like other mesograzers, employs algal hosts both as a food source as well as for shelter. Therefore, understanding the pressures exerted on *E. viridis* populations by biotic (e.g. predation) and abiotic (e.g. removal by wave force) drivers and assessing if algal hosts play a role in circumventing these pressures is an important undertaking. Our hypothesis is that the filamentous, prostrate morphology of *C. rupestris* provides better refuge from predation through crypsis and removal from waves than the planar, upright morphology of *C. fragile*.

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