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The influence of light and water flow on the growth and physiology of the scleractinian coral *Galaxea fascicularis*

Miriam Schutter

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The influence of light and water flow on the growth and physiology of the scleractinian coral *Galaxea fascicularis*

Miriam Schutter

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Miriam Schutter
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Chapter 1

General introduction

Miriam Schutter

1 Background

Scleractinian corals are the key organisms of tropical coral reefs and are, together with crustose coralline algae, responsible for building the carbonate reef structure that provides the physical structure and ecological habitat to thousands of other reef organisms. Coral reefs are "the tropical rainforests of the sea": the most biodiverse marine ecosystems in the world.

This intriguing and important ecosystem is threatened by both natural and anthropogenic factors such as global warming and ocean acidification (Hoegh-Guldberg et al. 2007), eutrophication (Szmant 2002), pollution, sedimentation, terrestrial run-off (Fabricius 2005), irresponsible dive tourism, overfishing (Scheffer et al. 2001; Jackson et al. 2001), destructive fishing (e.g. use of cyanide and blast fishing) and collection for aquarium trade (Bruckner et al. 2001). In the last couple of decades there has been an increasing number of bleaching events (Jones et al. 1997; Coles and Brown 2003), a large decrease in coral cover (Gardner et al. 2003; Bellwood et al. 2004) and an overall decline of biodiversity (Bellwood and Hughes 2001).

Concurrent with the gradual decline of coral reefs, there is a growing interest in keeping this delicate and beautiful ecosystem in aquaria by both hobbyists and public aquaria. The trade in marine ornamental species has become a multi million dollar industry. It is the mission of zoos and public aquaria to create more public awareness about the importance, beauty and sensitivity of the reef ecosystem. Therefore, to reduce additional harvesting pressure on the coral reef, it is the policy of zoos and public aquaria to display organisms that originate from sustainable breeding facilities. This policy has created an increased effort to develop cost-effective in situ (open sea) and ex situ (aquarium) culture of corals. To support this effort, the CORALZOO project was established and funded by the European Union (contract nr. 012547). In this project, universities and public aquaria collaborated to improve techniques for breeding and husbandry of scleractinian corals in closed aquarium systems (Osinga et al. 2005; Osinga 2007, 2008). This thesis reports research on factors controlling coral growth.

The relevance of this research project is two-fold. First, the deduction of universal mechanisms of coral growth can be used for sustainable aquaculture of scleractinian corals in public aquaria. Second, being successful in sustainable aquaculture of scleractinian corals indirectly contributes to reef conservation. Aquaculture is increasingly mentioned as a priority solution to reduce harvesting pressure on coral reefs (Parks et al. 2003; Hii et al. 2008). Moreover, the conservation of coral reefs is also ensured through safeguarding gene populations in aquaria for possible future restoration projects.

2 Closed aquarium systems

For a long time it was not possible to maintain living corals in closed aquarium systems, since corals are very demanding and sensitive to changes in their environment. Proper water chemistry is fundamental to keep corals alive and growing in aquaria (Wilkens 1973; Delbeek 2001). Obviously, this is less of a problem for open-system aquaria that have unlimited access to natural seawater. However, closed-system aquaria are often located far from the ocean and have to use artificial sea salt to make up their seawater. With the help of technological advances such as in the control of water chemistry, new lighting equipment and technologies for simulating water movement, it is possible to maintain corals ex-situ in a healthy condition for many years (Carlson 1999). Still, much knowledge on coral husbandry is anecdotal (e.g. reported in hobby magazines and popular books) and not scientifically based. A scientific approach is needed to validate and improve the common practice, and to reveal the underlying mechanisms responsible for well-being and growth.

Little is known yet about the impact of artificial environments upon scleractinian growth, morphology, calcification, behaviour and reproduction (Clode and Marshall 2003). Compared to the natural environment of coral reefs, closed aquarium systems are low in diversity of plants and animals, deficient in natural zoo- and phytoplankton communities and relatively rich in bacteria. Harvesting zooplankton from the sea to feed corals in closed aquarium systems is neither sustainable nor economically viable. Therefore, usually brine shrimp (*Artemia* nauplii) are used to feed corals in captivity (Hii et al. 2008).

Artemia are also widely used as live food for the larval culture of fish and shrimp species (Lavens and Sorgeloos 2000). Although *Artemia* nauplii are not the natural food source for corals, they are well-accepted as a food source by many different coral species (Hii et al. 2008; Houlbreque and Ferrier-Pagès 2009). Moreover, dormant *Artemia* cysts are commercially widely available, can be stored for long periods and can be easily cultured and enriched with different nutrients to meet specific nutritional needs.

Closed aquarium systems are generally high in nitrate and dissolved organic nutrients and require significant effort to maintain calcium, pH and alkalinity levels. The small size of most aquaria does not allow for much buffer capacity, which makes either frequent water changes or chemical addition of (trace)elements necessary. Chemical additions, on its turn, may lead to imbalances in sea water chemistry. Additionally, light intensity and water flow rates are generally quite low compared to most reefs, ultraviolet light is virtually absent, and the light field is usually unidirectional and unvarying, with no lunar cycles or seasonality (Carlson 1999, 2008). Clearly, closed aquaria systems are not exact simulations of nature. However, aquaria have the advantage of allowing a certain amount of control of (known) biological and/or abiotic factors. Therefore, aquaria are an ideal setting to study biological responses to environmental changes and factors controlling growth.

3 Biology of scleractinian corals

In order to understand coral growth, an introduction to the biology of scleractinian corals is needed. Scleractinian or reef-building corals are member of the phylum Cnidaria and are therefore closely related to other stinging animals such as jellyfish and sea anemones. In contrast to the solitary life form of anemones (being a single polyp), scleractinian corals are mostly colonial modular animals that consist of numerous polyps that are interconnected by tissue (Figure 1).

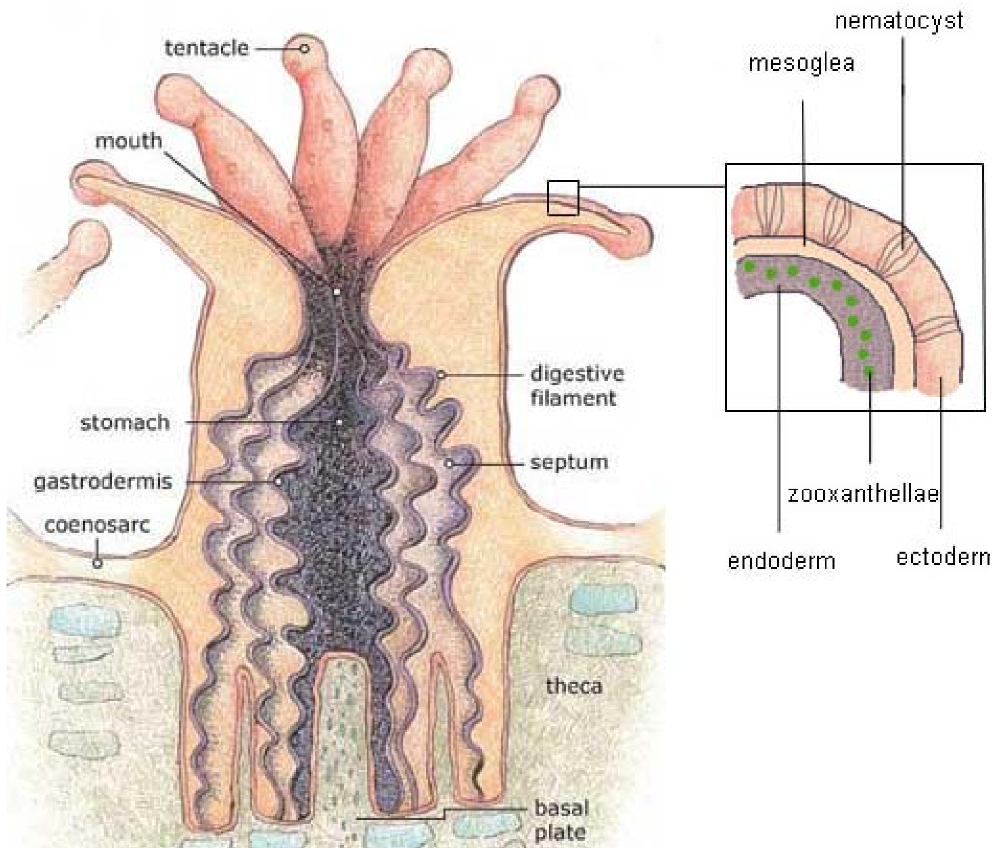

Figure 1: *Schematic drawing of a coral polyp. Note the different layers of coral tissue (ectoderm, mesoglea and endoderm) and the zooxanthellae that reside inside the endoderm (modified from original source: NOAA Ocean Service Education; http://oceanservice.noaa.gov/education/kits/corals/media/coral01a_462.jpg).*

Most reef-building corals live in symbiosis with unicellular dinoflagellates, also known as zooxanthellae (*Symbiodinium microadriaticum*). The zooxanthellae reside within perialgal vacuoles inside the endodermal cells of their coral host and supply their host with organic carbon that is produced by photosynthesis. Corals are mixotrophic in the sense that, besides this phototrophic feeding, they can also feed heterotrophically by capturing zooplankton and bacteria or taking up fine suspended particulate matter and dissolved organic matter (Anthony 1999; Ferrier-Pagès et al. 2003). Several ways of heterotrophic feeding are mentioned in literature, such as predation using nematocyst discharge, ten-

tacle grabbing, ciliary feeding using mucus entrapment, absorbance of dissolved organic matter, nutrient absorption from bacteria proliferating in mucus layer and possibly even digestion of zooxanthellae (Titlyanov and Titlyanova 2002a; Houlbreque and Ferrier-Pagès 2009).

The symbiosis allows for tight nutrient recycling which is a good adaptation for survival under the oligotrophic circumstances in which these corals are usually living. The photosynthetically active zooxanthellae use light energy for fixation of inorganic carbon into organic compounds such as sugars and glycerol. Inorganic nitrogen, such as ammonium (NH_4^+), and phosphorous (which are needed for synthesis of proteins by the zooxanthellae) are absorbed from the environment by the coral or recycled from coral waste products. The organic compounds produced by the zooxanthellae, also called photosynthates, are used first to satisfy their own metabolic needs, after which the remainder will be translocated to the coral host. It is estimated that circa 90% of the organic compounds produced by the zooxanthellae is translocated to the coral host (Davies 1984), but this varies between species. Once at the coral host, the photosynthates will either be respired, stored as lipid (Crossland 1980; Anthony et al. 2002) and/or excreted as mucus (Davies 1984; Crossland 1987; Brown and Bythell 2005). The oxygen produced during zooxanthellar photosynthesis is used for respiration and, if needed, additional oxygen is taken up from the environment (e.g. at night). Since photosynthates generally have a very high C:N ratio (i.e. are low in nitrogen), these compounds do not provide the necessary building blocks to support growth of the coral host. For this reason, these photosynthetic products are also called “junk food” (Dubinsky and Jokiel 1994). Mucus production and excretion was initially proposed to function primarily as an excretory pathway for excess organic carbon produced via symbiont photosynthesis, i.e. junkfood (Davies 1984). It is estimated that up to 45% of daily net photosynthates is being released as mucus and dissolved organic carbon (Davies 1984; Crossland 1987; Bythell 1988; Edmunds and Davies 1989). However, since the excretion of mucus seems not always a consequence of a low quality (high C:N) diet or excess production (Crossland 1987; Brown and Bythell 2005) other physiological and ecological roles are also proposed, such as a possible role in calcification, defense against damage by ultra violet radiation, defense against

smothering by sediment, defense against pathogens and defense against pollutants and other stresses (Brown and Bythell 2005). Nutrients necessary for coral growth, such as organic nitrogen and phosphorus, cannot be supplied by the zooxanthellae in sufficient amounts, but can be obtained by feeding heterotrophically (Ferrier-Pagès et al. 2003) or from absorption. See Figure 2 for a schematic overview of nutrient flows occurring within the coral-algal symbiosis that may be relevant to the coral growth.

The evolution of the coral-algal symbiosis resulted in integration and adaptation of both partners to each other and to the prevailing external circumstances. As a result, symbiotic organisms are not simply the sum of each organism separately, but an integrated whole (Yellowlees et al. 2008) that receives both benefits and constraints from their integration (Furla et al. 2005).

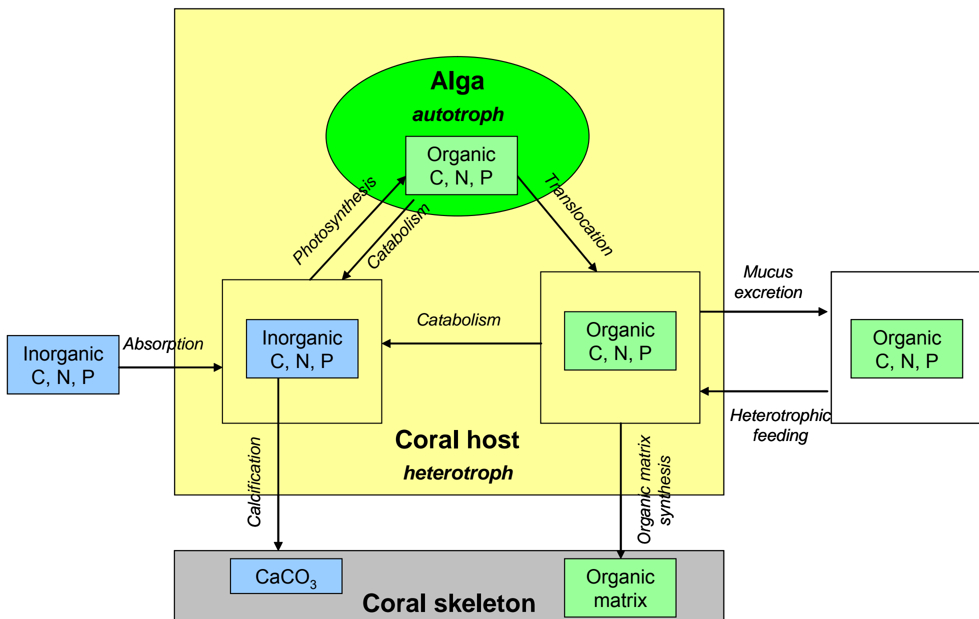

Figure 2: *Schematic illustration of cycling of organic and inorganic nutrients within the coral-algal symbiosis (i.e. holobiont). C=carbon, N= nitrogen, P= phosphate.*

Growth of scleractinian corals can be divided in three components: 1) skeletal growth due to the deposition of an external skeleton of calcium carbonate aided by the synthesis of an organic matrix in a process called calcification, 2) tissue growth and 3) growth of

zooxanthellae. Growth of scleractinian corals can predominantly be attributed to increasing skeletal mass, i.e. calcification. Prerequisites for coral calcification are: a) the supply of Ca^{2+} ions, b) the supply of an inorganic carbon source (either HCO_3^- taken up from water column or metabolic CO_2 from respiratory processes) c) a high aragonite saturation state (Ω) that favors precipitation of calcium carbonate, d) the supply of precursors for the organic matrix, either translocated by zooxanthellae (Muscatine and Cernichiari 1969; Pearse and Muscatine 1971; Young et al. 1971; Barnes and Crossland 1978; Richevich and Loya 1984) or supplied via heterotrophic feeding (Pearse and Muscatine 1971; Allemand et al. 1998a) and e) energy (ATP) for e.g. the uptake and transport of Ca^{2+} through different cellular layers.

According to the light-enhanced calcification theory (see Gattuso et al. 1999; Allemand et al. 1998b for review), the symbiosis with zooxanthellae is aiding to the process of skeletal growth. According to this theory, calcification of the coral host is enhanced by photosynthesis of zooxanthellae (Goreau and Goreau 1959; Pearse and Muscatine 1971; Allemand et al. 2004). Indeed, on average, calcification in light is found to be around three times higher than calcification in darkness (Gattuso et al. 1999). Although photosynthesis and calcification are spatially separated processes (photosynthesis occurs in the oral tissue layer and calcification in the aboral tissue layer), they do share a common pool of inorganic carbon inside the coelenteron of the coral host, accounting for the interaction between these two processes. The exact mechanisms of the enhancement of calcification by photosynthesis are still a matter of debate (Gattuso et al. 1999; Furla et al. 2000). The proposed hypotheses can be classified in two groups: 1) photosynthesis modifies the inorganic (carbonate) chemistry (pH and Ω) and 2) photosynthesis modifies the organic chemistry (e.g. ATP or organic matrix precursors) (see Gattuso et al. 1999; Allemand et al. 2004 for review). The importance of the organic matrix for the process of calcification is widely acknowledged (Allemand et al. 2004), just as the qualitative importance of the supply of organic matrix precursors by either zooxanthellar photosynthesis or through heterotrophic feeding. However, the quantitative importance of each source of precursors remains largely unknown. Possibly, the importance of heterotrophic feeding for the synthesis of the organic matrix is currently underestimated.

Growth and survival of scleractinian corals can be influenced by various biotic and abiotic factors (Table 1) and can be described in several ways, either directly by the result of the process of calcification (e.g. skeletal mass, surface area, polyp number), or indirectly by the process of calcification (e.g. calcium uptake, HCO_3^- depletion)

4 Environmental influences on coral growth

Coral growth depends on various endogenic and exogenic factors. The relation of coral growth with mixed environmental variables such as depth (Bosscher and Meesters 1993; Heiss 1994), latitude (Grigg 1982; Crossland et al. 1991; Logan and Tomascik 1991; Stimson 1996, Heiss and Dullo 1997, Harriott 1999; Dullo 2005) and season (Crossland 1984; Patzold 1984) have long been recognized. Growth rates of scleractinian corals generally decrease with increasing water depth. However, due to the mixed nature of this variable, this effect could be related to either light, water movement or resuspension of sediments and organic matter. Similarly, there are several potential factors controlling the decrease of growth rates with increasing latitude, such as decreasing temperature, photoperiod (day length) and irradiance. Light was assumed to be the primary factor controlling growth as a function of depth, while the decline in calcification rate with increased latitude was attributed to the effects of reduced temperature and, to a lesser extent, reduced light (Lough and Barnes 2000)
In laboratory or field manipulation studies, environmental factors influencing coral growth were studied separately, such as light (e.g. Marubini et al. 2001; Reynaud-Vaganay et al. 2001; Reynaud et al. 2004; Schlacher et al. 2007), water flow (Montebon and Yap 1997; Kuffner et al. 2001; Sebens et al. 2003; Nakamura 2005), temperature (e.g. Jokiel and Coles 1978; Crossland 1984; Marshall and Clode 2004), water quality (e.g. Marubini and Davies 1996; Fabricius 2005), aragonite saturation state (Gattuso et al. 1998, Marubini et al. 2001), and heterotrophic feeding (Ferrier-Pagès et al. 2003; Houlbreque et al. 2003, 2004; Houlbreque and Ferrier-Pagès 2009). Most of these studies involved short term manipulations of factors.

Table 1: *Known biotic and abiotic factors that affect the growth of scleractinian corals.*

Abiotic factor	References
Light (irradiance)	Falkowski et al. 1990
Water flow	Lesser et al. 1994
Water depth	Bosscher 1992; Bosscher and Meesters 1993
Latitude	Grigg 1982; Crossland et al. 1991; Logan and Tomascik 1991; Stimson 1996; Heiss and Dullo 1997; Harriott 1999; Dullo 2005
Season	Crossland 1984; Patzold 1984
Temperature	Jokiel and Coles 1990; Marshall and Clode 2004
Turbidity	Anthony and Fabricius 2000
Sedimentation	Rogers 1990
Water quality	
Aragonite saturation state	Gattuso et al. 1998; Kleypas et al. 1999; Langdon et al. 2000; Leclerq et al. 2000; Ohde and Hossain 2004; Schneider and Erez 2006
pH	Marubini and Atkinson 1999, Fine and Tchernov 2007
Alkalinity and associated components	Marubini and Thake 1999; Marubini et al. 2008
Nutrients (nitrate, phosphate)	Marubini and Davies 1996; Ferrier-Pagès et al. 2000; Fabricius et al. 2005
Trace elements (Co, Zn, Fe)	Ferrier-Pagès et al. 2001; FerrierPagès et al. 2005
Oxygen concentration	Rinkevich and Loya 1984
Biotic factors	**References**
Nutrition/ Heterotrophic feeding	Ferrier-Pagès et al. 2003; Houlbreque et al. 2003, 2004; Houlbreque and Ferrier-Pagès 2009
Intraspecific competition	Rinkevich and Loya 1985
Interspecific competition	Tanner 1995
Predation	Miller and Hay 1998
Coral disease	Richardson 1998; Lesser et al. 2007

Although the mechanisms of calcification are still not completely understood (Furla et al. 2000; Moya et al. 2008a), based on the fundamental requirements for calcification some more specific hypotheses can be postulated regarding the environmental controls of coral growth. Provided that the primary conditions (e.g. light, temperature, salinity) for coral well-being are fulfilled, parameters that will directly favor the process of calcification should:

1. increase the supply and/or availability of inorganic carbon, such as high alkalinity or aragonite saturation state of the seawater, a higher water flow (HCO_3^-) and/ or a higher respiration rate (metabolic CO_2),
2. increase the availability of calcium (Marshall and Clode 2002),
3. favor the precipitation of calcium carbonate, such as an increased pH and aragonite saturation state near the site of calcification (i.e. the coelenteron). The currently most probable mechanism proposed in literature involves the photosynthetic release of OH^- which neutralizes H^+ produced in calcification (Allemand et al. 2004),
4. increase the supply of precursors for the organic matrix, both via algal photosynthetic products (Muscatine and Cernichiari 1969; Pearse and Muscatine 1971; Young et al. 1971; Barnes and Crossland 1978; Richevich and Loya 1984) and via heterotrophic feeding (Pearse and Muscatine1971; Allemand et al. 1998a),
5. increase the energy available for processes such as calcium transport (Tambutté et al. 1996), organic matrix synthesis (Wainwright 1963; Chalker and Taylor 1975) and calcification, such as energy from photosynthesis and heterotrophic feeding, and
6. influence the functioning of enzymes, such as temperature. Important enzymes are those which are needed for the transport and/or conversion of HCO_3^- to CO_2 and the other way round (i.e. carbonic anhydrase).

Different environmental factors will influence the different requirements for the process of calcification (1 through 6) in different ways, and they might also interact.

4.1 Water flow

The importance of water flow for coral well-being and coral growth is often underestimated (Borneman 2008). Water flow has many positive effects on coral biology. First, it reduces the diffusive boundary layer around the coral (Shashar et al. 1996), thereby facilitating mass transfer (i.e. supply/uptake and removal/excretion) of ions (Ca^{2+}, HCO_3^-, $NH4^+$), dissolved organic matter (e.g. DOC) and gasses (supply of CO_2 and removal of O_2). Water flow is therefore critical in optimizing metabolic rates, such as respiration, photosynthesis and uptake of HCO_3^- for calcification. Second, it increases the encounter rate of suspended particles for sedentary animals such as corals and is therefore involved in the level of heterotrophic feeding. Third, it aids in the removal of sediment and mucus from the coral surface, thereby preventing suffocation.

It is hypothesized that the absence of flow will be detrimental to both growth and survival of corals, since it will impede any exchange with the environment (see Figure 3a). Observations from natural coral reefs indicate a correlation between reduced water flow and coral bleaching (Lesser et al. 1997; Nakamura and Van Woesik 2001; Nakamura et al. 2003). Higher flow speeds will fulfill different requirements for growth by influencing different parameters of the nutrient budget, such as absorption of solutes, respiration (catabolism) and photosynthesis (through the uptake of gasses), translocation of photosynthates as a result of increased photosynthetic rates and uptake of organic food particles (through increased encounter rate of food particles) (Figure 3b,c). It is not known whether water flow has an effect on mucus production. At low irradiance, the importance of mucus production as a component of the nutrient budget is expected to be negligible.
By stimulation of both photosynthesis and respiration on the one hand and heterotrophic feeding on the other hand, increasing water flow will stimulate skeletal growth both through enhancement of calcification and enhancement of organic matrix synthesis. The extent of the effect of water flow on coral growth will obviously depend on nutrient concentrations, availability of food and light regime. The relationship between increasing water flow and coral growth is expected to follow a hyperbolic function: First, increasing water flow will

first lead to enhanced coral metabolism and subsequent improvement of growth, until second, mass transfer will no longer be limiting and growth will reach its maximum, and third, water flow becomes damaging.

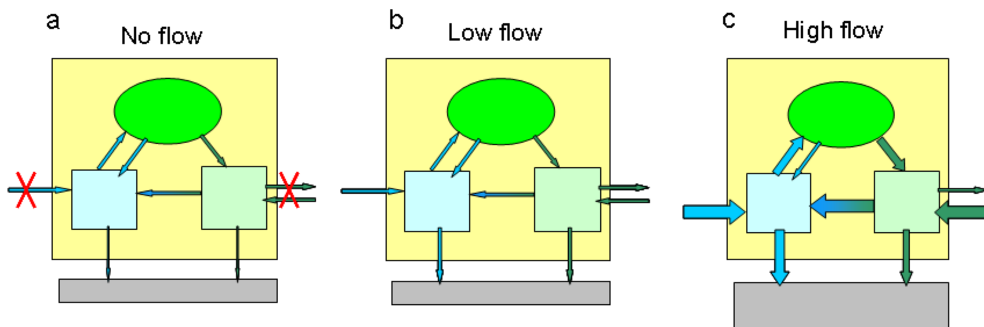

Figure 3: *Schematic overview of the various inputs and outputs in the coral nutrient budget (left side: inorganic (blue), right side: organic (green), and hypothesized changes between no flow (a), low flow (b) and high flow conditions (c) (see text).*

4.2 Light

Since growth and distribution of scleractinian corals is limited to the euphotic zone of the coral reef, the importance of light for keeping corals in aquaria is often stressed. Light has many effects on coral biology: First, it enhances photosynthesis according to a hyperbolic tangent function (Chalker 1981). This means that at low irradiance, the rate of photosynthesis is nearly directly proportional to irradiance. At higher irradiance, the rate of photosynthesis rapidly approaches a horizontal asymptote, which is the point where saturation of photosynthesis is reached (the maximum gross photosynthetic rate, P^g_{max}). At relatively high irradiance levels, increasing irradiance will result in photoinhibition of photosynthesis and consequently reduced photosynthetic rates (Smith et al. 2005).

Second, calcification is enhanced by photosynthesis of the zooxanthellae. The relationship between light and calcification can be described by the same relationship as photosynthesis (Chalker 1981). As mentioned before, the mechanism of enhancement of calcification by photosynthesis is still a matter of debate (Moya et al. 2006, 2008a). Third, both dark and light respiration are enhanced by increasing photosynthesis (Harland and

Davies 1996; Al-Horani et al. 2003). Dark respiration is postulated to be enhanced by the presence of more translocated photosynthates, while light respiration is postulated to be enhanced by the presence of photosynthetically produced oxygen. This leads to a higher internal carbon cycle (Al-Horani et al. 2003b) and a higher availability of ATP for energy-consuming processes such as calcium transport (Tambutté et al. 1996) and organic matrix synthesis (Chalker and Taylor 1975; Palmer 1983). Additionally, higher respiration rates result in the presence of a larger amount of metabolic CO_2 that can be used for calcification. Fourth, as a result of higher photosynthetic rate, the amount of photosynthates that is translocated is also increased (about 90-95%; Davies 1984; Muscatine et al. 1984). These photosynthates might be used as a precursor of the organic matrix (Muscatine and Cernichiari 1969; Pearse and Muscatine 1971; Young et al. 1971; Barnes and Crossland 1978; Rinkevich and Loya 1984), thus simultaneously stimulating calcification. Fifth, light plays a central role in mucus production (Crossland 1987; Brown and Bythell 2005), which increases with irradiance. The effect of mucus production on calcification is not known, although a possible role for mucus in calcification is suggested (Brown and Bythell 2005).

Therefore, an increase in irradiance will positively influence different parameters of the carbon budget: photosynthesis and respiration, calcification (i.e. precipitation of $CaCO_3$), translocation of photosynthates, organic matrix synthesis and mucus production (Figure 4a,b). Concurrently, different requirements for calcification are fulfilled: photosynthesis will optimize both the inorganic and organic requirements for calcification (i.e. aragonite saturation state at site of calcification and organic matrix precursors), respiration will increase both the supply of inorganic carbon (metabolic CO_2) and the supply of energy for calcification and the transport of calcium. The extent of this effect will obviously depend on nutrient concentrations, availability of food and flow regime.

Although the presence of light has a positive effect on calcification, it is not known whether light is truly beneficial to coral growth throughout the entire light range (up to 2000 $\mu E\ m^{-2}\ s^{-1}$ in the field, Mass et al. 2007). Application of such high light intensities in the aquarium is technically quite demanding, if not impossible, using standard available arti-

ficial light sources. However, it remains possible to study how skeletal growth increases with irradiance and whether such an increase is mediated by photosynthesis. Assuming light-enhancement of calcification through photosynthesis, it was hypothesized that coral growth and net photosynthesis are linearly correlated.

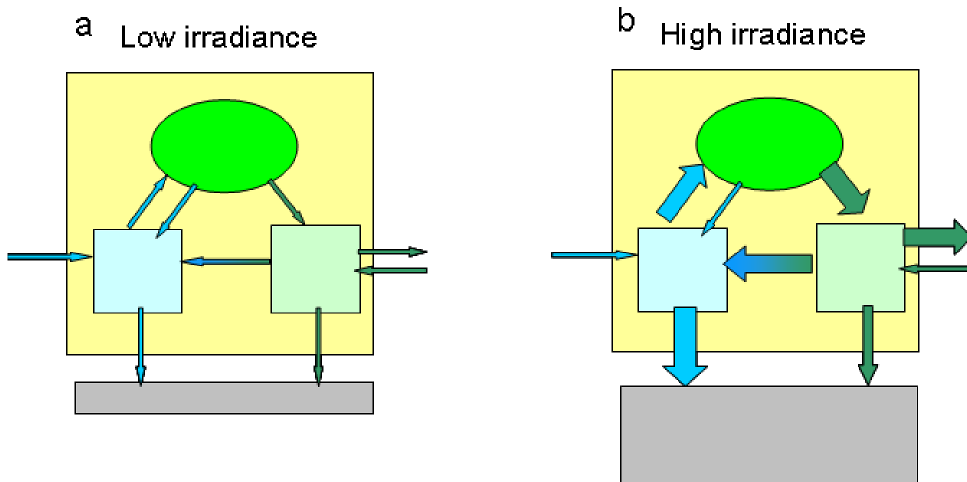

Figure 4: *Schematic overview of the various inputs and outputs in the coral nutrient budget (left side: inorganic (blue), right side: organic (green), and hypothesized changes between low irradiance (a) and high irradiance (b) (see text).*

In addition, light can be applied in different ways. The quantity of light (or photons) that is available for the zooxanthellae is not only determined by photon flux density, but also by the length of the photoperiod. Light duration has been identified as an important factor influencing coral growth. However, in nature, light duration varies concurrently with temperature and irradiance (i.e. with latitude or with season), making it difficult to separate these factors. Thus, although corals seem to grow faster around the equator (i.e. at low latitude; Crossland 1981; Stimson 1996; Harriott 1999) and during summer time (Shinn 1966; Patzold 1984), it is not possible to determine the relative contribution of each factor based on such studies. However, in aquaria it is possible to study these factors separately. Photoperiod is an important factor to consider for the culture of corals in aquaria, since – next to irradiance – it can determine part of the cost-efficiency of coral culture (energy input versus coral growth). It is hypothesized that a longer light duration and

consequently more hours of light-enhanced calcification will increase daily growth rates, provided that the hourly calcification rate and photosynthetic rate remain unchanged.

4.3 Synergistic effects of irradiance and water flow

Irradiance and water flow are proposed to have synergistic effects. At higher irradiance, increased photosynthesis will demand: 1) a higher supply of inorganic carbon, 2) a higher removal rate of accumulating oxygen, and 3) a higher removal rate of mucus (to prevent intoxication). All of this can be taken care of by sufficient water flow. The extent of the effect of either parameter depends on the other. Moreover, both parameters affect coral growth in a different ways. Increasing irradiance increases the supply of organic matrix precursors for organic matrix synthesis through photosynthesis and optimizes inorganic carbonate chemistry for calcification, while increasing water flow increases the supply of organic matrix precursors through increases supply of food particles and increases the availability of inorganic carbon for calcification by increasing respiration. Thus, together, irradiance and water flow can cover all the parameters of the nutrient budget and requirements for coral growth, depending on external (in)organic nutrient concentrations and availability of food.

It is hypothesized that light and water flow have a strong interacting effect on coral growth, since water flow is postulated to become more important at high irradiance levels. At low flow, coral growth may be inhibited at high irradiance, due to the accumulation of oxygen (i.e. oxidative stress) and the reduced interaction with the environment (e.g. absorption, excretion) (Figure 5). Although also other factors seem to be important, it is expected that photosynthesis might be an explanatory variable for the effect of light and water flow on coral growth.

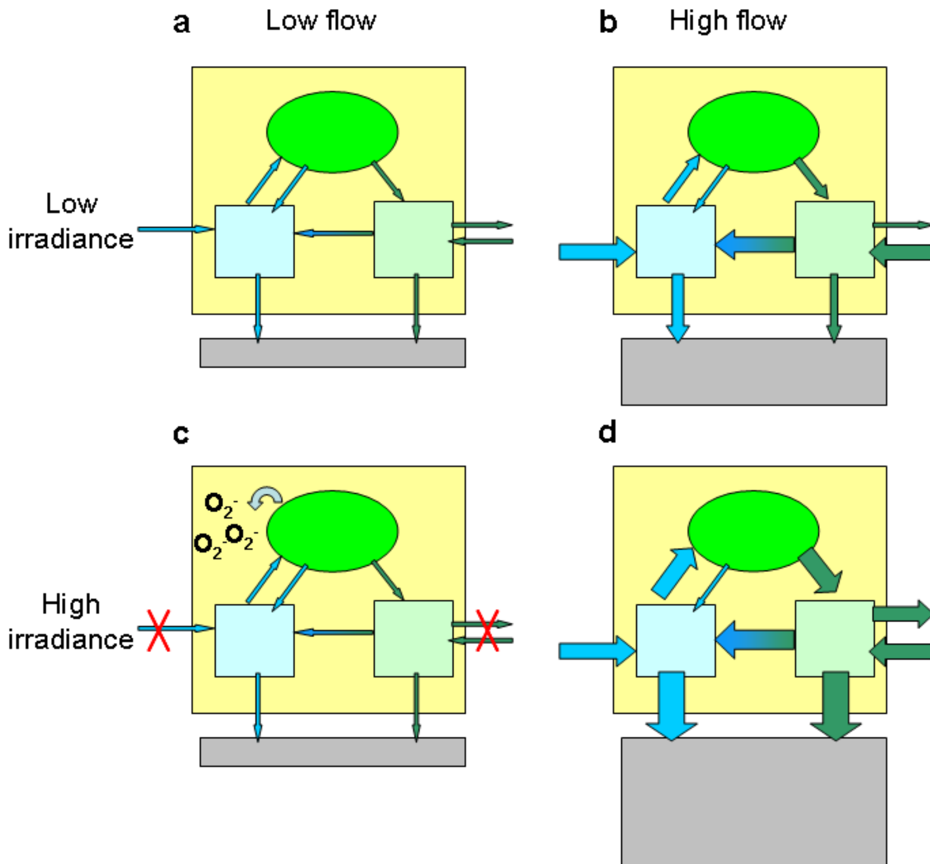

Figure 5: *Schematic overview of the various inputs and outputs in the coral carbon budget (left side: inorganic (blue), right side: organic (green)), and hypothesized result under low flow x intermediate ight (a), high flow x intermediate light (b), low flow x high light (c) and high flow x high light (d) conditions (see text).*

5 Aim and objectives of this thesis

The aim of this thesis is to create scientific knowledge on the eco-physiology of coral growth, which can contribute to sustainable aquaculture of scleractinian corals in public aquaria. Due to the growing interest in tropical coral reefs, there is an increasing demand for marine ornamental species, such as scleractinian corals, for the aquarium trade. To meet this demand, and concurrently reduce harvest from the wild, sustainable culture

of coral has to be warranted. However, much of the information on the aquaculture of scleractinian corals is still anecdotal and not scientifically based. The influence of abiotic factors and the mechanisms of coral growth are probably best studied by using existing knowledge from the field and anecdotal knowledge from aquarium hobbyists, and to combine this knowledge for the design of scientific experiments to systematically determine the response to each (combination of) important factor(s). In this thesis, the influence of light (irradiance and photoperiod) and water flow on coral growth was studied. The Indo-Pacific coral species *Galaxea fascicularis* was chosen for this study, since this species is easy to grow and since it is very easy to prepare coral nubbins (single polyp clones) of this coral species due to the large size of its polyps.

The objectives of this thesis are:

1) to study the effect of water flow on the growth and physiology of *G. fascicularis* (**Chapter 2**)
2) to study the effect of irradiance on growth of *G. fascicularis* and to relate this growth to photosynthetic rate (**Chapter 3**)
3) to study the effect of photoperiod on growth and photoacclimation of *G. fascicularis* (**Chapter 4**)
4) to study the interaction between light and water flow for growth and physiology of *G. fascicularis* (**Chapter 5**)
5) to review factors controlling coral growth and assess the economic potential of coral farming (**Chapter 6**)

A better understanding of how these abiotic factors influence coral growth will improve the aquaculture of scleractinian corals and aid in the design of sustainable and cost- efficient coral culture systems.

6 Outline of the thesis

The focus of this thesis is to study how light and water flow affect the growth of the scleractinian coral *G. fascicularis* in closed aquarium systems. This thesis is composed of a general introduction (**Chapter 1**), four experimental chapters (**Chapter 2, 3, 4** and **5**), one review chapter on the biology and economics of coral growth (**Chapter 6**) and a final discussion with future perspectives (**Chapter 7**). The effect of light and water flow on growth and physiology is first studied separately and then together using a factorial design.

In **Chapter two**, the influence of water flow on coral growth and physiology is studied. This chapter also analyzes the growth kinetics of *G. fascicularis*. In **Chapter three**, the influence of light on coral growth is studied and correlated to a photosynthesis-irradiance curve. This enables us to say more about light-enhanced calcification throughout a range of light intensities. In **Chapter four**, it was aimed to study the influence of photoperiod on coral growth and physiology. However, since growth during this experiment was not limited by irradiance, our data were used to study the mechanism of photoacclimation of *G. fascicularis* under light-saturating conditions. In **Chapter five**, the interaction between light and water flow on coral growth and physiology was studied. **Chapter six** reviews the biology and economics of coral growth, summarizing factors that either stimulate, limit or inhibit coral growth and how this knowledge can contribute to economical coral farming. Finally in **Chapter seven**, the overall results obtained during this thesis are discussed. A synthesis is made of the underlying mechanisms of coral growth and which factors are critical for optimizing coral growth in closed aquarium systems. Besides a critical reflection on the past four years of research, also directions for future research are given.

Chapter 2

The effect of different flow regimes on the growth and metabolic rates of the scleractinian coral *Galaxea fascicularis*

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Abstract

To study the effect of water flow on coral growth, four series of ten coral nubbins of *Galaxea fascicularis* were exposed to four different flow regimes (0 cm s^{-1}, 10 cm s^{-1}, 20 cm s^{-1} and 25 cm s^{-1}, bidirectional flow) for 42 weeks. Buoyant mass, surface area and polyp number were measured at regular intervals. Net photosynthesis and dark respiration were measured at the corresponding flow speeds and daily amount of photosynthetic carbon left for coral growth was calculated. Finally, skeletal density and CN content, chlorophyll concentration and dry mass of coral tissue were determined for each coral.

Specific growth rate (in day^{-1}) decreased with time in each flow treatment. Absence of flow resulted in significantly lower growth rates. Average specific growth rate was not significantly different between 10 and 20 cm s^{-1}, while it was significantly higher at 25 cm s^{-1}. However, differences in growth between treatments were not consistent between growth intervals. From 10 to 25 cm s^{-1}, average net photosynthetic rate decreased and average dark respiration rate increased. Scope for growth based on phototrophic carbon decreased with increasing flow.

Growth was not positively correlated with either photosynthesis, respiration or scope for growth. It is suggested that higher flow rates reduce the chance of disturbance of coral growth by competing algae, allowing corals to grow more often with the maximum specific growth rate possible under the given environmental conditions. Also other effects of increased flow, such as increased respiratory rates and increased (in)organic nutrient uptake, might have been co-responsible for the increased growth of the corals in 25 cm s^{-1}.

Keywords: *coral growth, water flow, photosynthesis, respiration,* Galaxea fascicularis

1. Introduction

Water flow is one of the most important abiotic factors influencing the growth of sedentary marine invertebrates (Sebens 1987). Particularly interesting is the effect of flow on the growth and metabolism of zooxanthellate scleractinian corals, due to the complex physiology of these animals. Zooxanthellate scleractinian corals live in symbiosis with unicellular algae, known as zooxanthellae, that translocate part of the carbon that is fixed during photosynthesis to their animal host. This symbiotic relationship allows the coral to benefit from both heterotrophic and phototrophic carbon sources.

The importance of water flow for different aspects of coral biology has received considerable attention. Water flow affects physiological processes such as photosynthesis and respiration by relieving diffusion limitation for dissolved gasses (Dennison and Barnes 1988; Patterson et al. 1991; Patterson 1992; Atkinson et al. 1994; Lesser et al. 1994; Shashar et al. 1996; Bruno and Edmunds 1998). Flow also affects the encounter and ingestion rate of food particles (Helmuth and Sebens 1993; Sebens 1997; Sebens et al. 1997, 1998), the uptake of dissolved inorganic nutrients such as nitrate, phosphate (Stambler 1991; Atkinson and Bilger 1992; Thomas and Atkinson 1997) and the uptake of inorganic carbon (Lesser et al. 1994). Third, flow aids in removal of harmful waste products such as oxygen radicals or its derivatives (Nakamura and Van Woesik 20015) and in removal of sediments or nuisance algae that might otherwise suffocate the coral (Rogers 1990; Anthony and Fabricius 2000; Box and Mumby 2007). On the other hand, water flow can also have negative effects on coral biology, for example by stressing the coral (Jokiel 1978) by damaging the delicate coral tissue, by breaking off branches of skeleton, or by restricting particle capture due to deformation and flattening of the tentacles (Sebens et al. 1997). Growth rates of corals will thus be determined by the sum of effects that flow exerts on coral physiology. The different processes affected by flow (i.e., feeding efficiency, gas exchange, waste removal) may each have their optimum at a different flow rate. Furthermore, optimal flow rates may vary among species and even among conspecific individuals. Indeed, some corals have been found to grow more rapidly when flow increases (Jokiel 1978; Montebon and Yap 1997; Nakamura and Yamasaki 2005),

while other corals were found to have comparable growth rates (Sebens et al. 2003) or even decreased growth rates (Kuffner 2001). Khalesi et al. (2007) found a hyperbolic profile of growth with increasing flow rate for a soft coral.

Most studies on the effects of flow on either growth or metabolic rates often examined only “low” and “high” flow treatments and did not characterize their flow regimes with a meaningful number for flow speed, or they examined only a limited range of flow speeds (e.g., Sebens et al. 2003, 0-10 cm s^{-1}). Besides that, different coral species – that might respond differently to flow speed - were used in previous studies, thus making it difficult to compare results. Therefore, it is hard to deduce optimal flow regimes for corals from the available data.

The first aim of this work was to study the effect of water flow on the (skeletal) growth of *Galaxea fascicularis*. For this, series of ten genetically identical coral nubbins of *G. fascicularis* were cultured for a period of 42 weeks at a wide range of defined flow speeds (0-25 cm s^{-1}) in a controlled aquarium environment. Growth was measured at regular intervals. It was expected to find a positive relationship between increasing water flow rates and skeletal growth. Secondly, effect of flow on photosynthesis, respiration, and the calculated daily amount of photosynthetic carbon left for coral growth were studied and related to effects of flow on growth. It was expected to find a positive correlation between phototrophic metabolism and growth. And third, at the end of the long-term and short-term experiments, the effect of water flow on different coral biomass parameters (CN content, chlorophyll concentration and ash-free dry mass of coral tissue and skeletal density) was determined. Here, we expected to find that corals cultured at higher flow regimes (i.e., higher hydrodynamic stress) would have a more dense skeleton, in order to withstand physical damage (Schuhmacher and Plewka 1981; Scoffin et al. 1992; Bucher et al. 1998).

2. Material and Methods

2.1 Long-term growth experiment

2.1.1 Study species

Fourty (40) coral nubbins (single polyp clones) of *G. fascicularis* were created of coral colonies that were grown at an irradiance of ca 60 µE m^{-2} s^{-1} (70W HQI) in a closed-circuit coral aquaculture system in Burgers Ocean, Arnhem, The Netherlands. This 6800 l system consists of four 1300 l aquaria and two 800 l sumps, one with live rock and the other without. The circulation system cycles 18 m^3 h^{-1} and the system is connected to a trickle tower, a 23.5 l self made Ca^{2+} reactor, and a Schuran Aquafloater AQ250 protein skimmer. Each coral nubbin was fixed to a 7x7cm PVC plate using a two component epoxy (Reef Construct, Aquamedic). To our knowlegde, no adverse effects of epoxy on coral growth have been reported. After a three week acclimation period, each coral nubbin was placed on one of four rectangular pegboards in two rows of five coral nubbins.

2.1.2 Experimental setup

Each pegboard containing 10 coral nubbins was assigned to each of four experimental treatments: no water flow (± 0 cm s^{-1}), ± 10 cm s^{-1} bidirectional water flow, ± 20 cm s^{-1} bidirectional water flow and ± 25 cm s^{-1} bidirectional water flow. The actual average flow speeds during the long-term growth experiment were resp. 1.2 ± 1 S.D. cm s^{-1}, 9.0 ± 2.5 S.D. cm s^{-1}, 17.5 ± 3.7 S.D. cm s^{-1} and 24.5 ± 4.4 S.D. cm s^{-1}. The different flow regimes were created using Tunze Turbelle Stream 6000 and 6100 pumps that were hung at either end of a submerged, open flowcell and adjusted to the desired flow speed (Figure 1). Flow direction was changed every 5 minutes using a Tunze 7095 Multicontroller. By offering water flow in two directions, it was assumed that the effect of flow on coral physiology would not be one-sided. Flow straighteners were constructed of 10 cm long PVC pipes with a diameter of 1 cm and placed downstream from the pump outlets and before the location of the corals, to create a more or less laminar flow. A rectangular cell was constructed for the 0 cm s^{-1} flow regime.

Flow speeds were measured every 4 weeks by placing a a SENSA RC-2 electro-magnetic velocity meter (Aquadata) in each submerged flowcell (in the absence of any corals) with its sensor tips 5 cm from the flow straighteners (Figure 1), which is the location of the first coral on each PVC plate. Flow speeds were adjusted if needed.

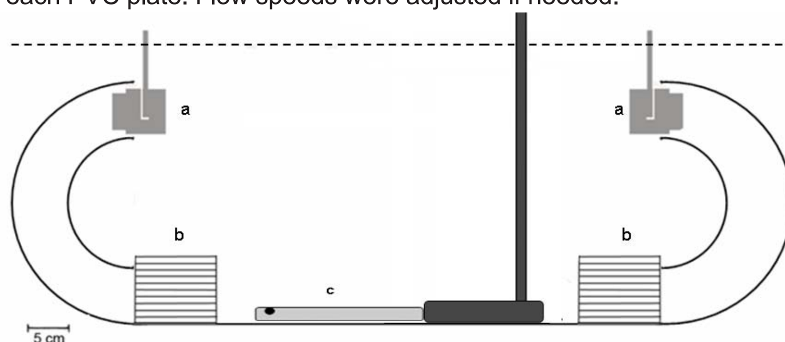

Figure 1: *Side view of a submerged flowcell (l x w x h: 95 x 30 x 25 cm). a: Tunze pumps, b: flow straigtheners, c: position of SENSA RC-2 electro-magnetic velocity meter (Aquadata) when measuring water flow. The small black circle is the position of the sensor tip. The dashed line indicates the water level inside the coral culture system.*

Lighting was provided by fluorescent T8 lighting systems with 36W Philips TI-D90 965 color bulbs, providing an equal light distribution (see Schutter et al. 2008). Irradiance was measured using a Li-Cor 192SA quantum underwater sensor and maintained at 90 µE m^{-2} s^{-1}. A light dark cycle of 10L:14D was applied.

Each experimental treatment was fed indirectly by daily feeding of the entire coral culture system (4-8 *Artemia* ml^{-1}) and additionally twice a week directly inside each experimental treatment (i.e., each submerged flowcell) (5000 ± 800 *Artemia* nauplii per treatment, yielding 15 *Artemia* l^{-1}). *Artemia* nauplii (Salt Lake aquafeed) were hatched on site and subsequently enriched using Rich Advanced feed for 24 hours.

Seawater was made up from Tropical Marine salt (Zoomix without bromide). Temperature was maintained at 26 ± 2 SD °C, salinity at 34 ± 0.3 SD ppt and pH at 8.0 ± 0.3 SD. Water quality parameters were measured at regular intervals. During the experiment, alkalinity in the system was 4.0 ± 1.0 SD mEq l^{-1}, calcium concentration 395 ± 20 SD mg l^{-1}, magnesium concentration 1200 ± 50 SD mg l^{-1}, nitrate concentration 0.03 ± 0.01 SD mg l^{-1} NO_3^- and phosphate concentration 0.02 ± 0.01 SD mg l^{-1} PO_4^{3-}.

2.1.3 Growth parameters and analysis of growth kinetics

Growth was measured as an increase in buoyant mass, surface area and polyp number of the coral nubbins. Determination of buoyant mass (using the so-called " the buoyant weighing technique") is a good method to measure skeletal growth, since coral tissue has a density which is similar to that of seawater and therefore does not contribute significantly to the buoyant mass of the coral. Tissue only comprises 1% of the total buoyant mass when tissue does not penetrate deep into the skeleton (Davies 1989). Moreover, buoyant weighing is a simple and non-destructive technique, allowing long-term monitoring of skeletal growth. It should be noted that buoyant mass is not a good approximation of skeletal mass, since the net upward buoyancy force (i.e. the weight of seawater displaced by the coral) will result in measuring only a fraction of the absolute mass (i.e. a fractional mass). To convert buoyant mass into skeletal mass, one needs to know the density of the seawater and the volume of the coral. Moreover, one needs to assume a constant density of the coral skeleton during the experiment. However, for the purpose of this study, knowledge of the magnitude of buoyant mass was sufficient to calculate growth rates.

Buoyant mass was measured in the laboratory by suspending each coral (plus PVC plate) on a hook in a defined volume of seawater at a constant depth. Seawater was maintained at 26°C and 34 ppt salinity. The hook was attached to an underweighing analytical balance (Kern&Sohn D-72458 Albstadt, type 870-13) using a thin nylon string (Osinga et al. 1999). Buoyant mass of each coral was measured and the average of three measurements was taken. The initial mass of the nubbins before their attachment to their PVC-plate at t=0 was estimated by weighing 5 similar-sized nubbins of a *G. fascicularis* colony on a weighing glass and taking the average. Using this parameter, it was possible to estimate the mass of the PVC plate and the amount of Reef Construct that was used to attach each coral to its plate. All our buoyant masss were corrected for this mass in order to obtain the buoyant mass of the coral colony itself. This mass was used as parameter for data analysis.

Surface area was measured as projected surface area. Pictures were taken perpendicular to the coral directly inside the aquarium system using a Nikon Coolpix S1 5.1 mp digital

camera in a Nikon WP-CP5 underwater housing. Surface area was determined by image analysis using ImageJ (1.37v) by tracing the live part of the coral colony. Since tentacle extension is variable over time, no tentacles that were extending beyond the skeleton were traced for surface area. Polyp number was counted visually. Only live polyps were counted. Newly formed polyps were only counted once they started projecting from the basal skeletal plate.

Buoyant mass and polyp number were determined every six weeks, while surface area was determined every three weeks for a 42 week period. For comparison with growth rates from a previous study (Schutter et al. 2008), exponential growth was assumed and specific growth rates (μ) were calculated using the formula:

$$\mu = (\ln BW_n - \ln BW_{n-1})/\Delta t \qquad [day^{-1}]$$

where μ is the specific growth rate (day^{-1}), BW_n is buoyant mass at the end of a growth interval, BW_{n-1} is buoyant mass at the start of a growth interval and Δt is time between measurements of buoyant mass in this growth interval. The same was done for surface area and polyp number.

2.2 Short term physiological experiments

2.2.1 Respirometric flowcell

A respirometric flowcell (1616 ± 5 ml) (Figure 2) was designed and built at Wageningen University to study the metabolic rates of *G. fascicularis* colonies in response to different water flow speeds. Water flow is created by two RC-280 model boat propellers (Ø 3 cm) that are driven by two separate Maxon DC motors that allow precise control of rotation speed through the EPOS_UserInterface (Version 2.31) software. The propellers are placed in such a way that they create an unidirectional flow inside the flowcell. Using this setup, flow speeds from 0 cm s^{-1} to 30 cm s^{-1} can be attained. Upstream from the coral colony, the water passes a 3 cm long flow straightener constructed from plastic straws (Ø 5 mm) to create a less turbulent flow. Behind the flow straighteners, a small coral colony

can be placed underneath a translucent lid made of Perspex, which allows passage of all wavelengths between 400-700 nm. Oxygen measurements are made using a LDO probe (Luminiscent Dissolved Oxygen, Hach) which is placed downstream of the coral colony. A built-in water jacket is connected to a TECO TR10 cooler to keep the water temperature inside the flowcell at 26 ± 0.4°C.

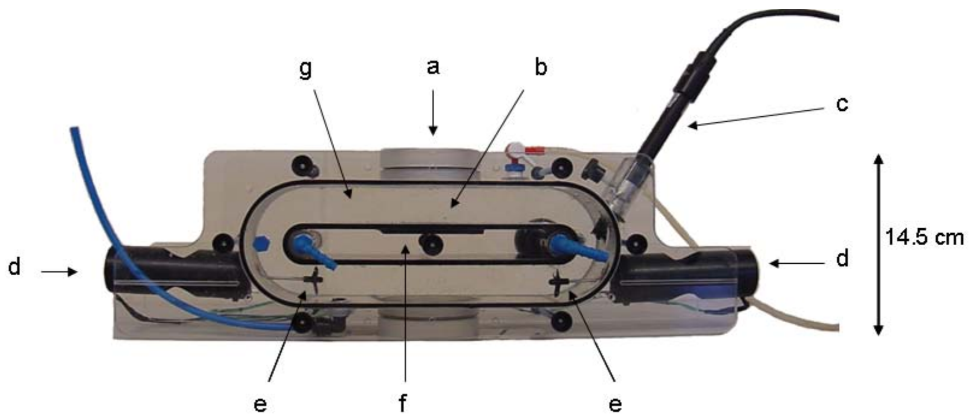

Figure 2: *Respirometric flow cell (version I). The most important parts are indicated with an arrow and acompanying alphabetic letter. a: glass screw cap, b: location of coral, c: location of oxygen probe (downstream of coral), d: site of attachment for the motor block that powers the propeller, e: propeller, f: internal water jacket that can be connected to a waterbath, g: location of flow straighteners (upstream from coral).*

Water flow speeds across the coral section were calibrated by tracing plastic particles (Ø 1 mm) moving in a 5 mm light beam plane created by a slide projector with a slitted cover across the lens. Video recordings were made using a JVC GR-DVL digital video camera and particle positions were traced in successive video frames (1/30 s apart) using Midas Player 2.2.0.8 (Xcitex, free version). Only particles that remained in the beam plane for 4 or more successive frames were used, to reduce error due to particles moving diagonally across the beam plane (Sebens and Johnson 1991).

2.2.2 Respirometric flow cell incubations

Three corals from each experimental flow treatment were randomly chosen at the end of the long-term growth experiment and used to measure photosynthetic and respiratory rates at their corresponding flow speeds. During the execution of these short-term experiments the experimental flow treatments in the coral culture system were maintained as before. It was not possible using our setup to get accurate oxygen reading for the corals from the zero flow regime.

Each coral was measured on three different days, each day starting with a control incubation in the light, three replicate 30 minute trials for photosynthesis, a control incubation in the dark and three replicate 30 minute trails for respiration. Net photosynthetic production of oxygen and respirometric consumption of oxygen was measured according to Schutter et al. 2008. Temperature inside the respirometric flowcell was maintained at 26 ± 0.5 SD °C and salinity at 34 ± 0.1 SD ppt.

Lighting was provided using a T5 lighting system (ATI) containing four 24W Aquablue Spezial bulbs. Irradiance was adjusted to an irradiance of ca 90 µE m^{-2} s^{-1} using a Profilux II aquatic computer-controller (GHL) and measured using a Li-Cor 192SA quantum underwater sensor. This irradiance corresponded to the irradiance experienced in the growth experiment.

Surface area, volume and buoyant mass of the experimental corals were determined weekly during this experimental period (see section 2.1.3). Surface area was determined in order to normalize the respirometric data. The volume of the coral was determined using the water displacement technique in order to correct flowcell volume for the space taken in by the coral. Buoyant mass was determined to monitor the growth during this experimental period. Besides that, buoyant mass of all corals from the growth experiment was determined before and after the respirometric flow cell incubations to test whether the extra experimental handling had an effect on growth rates of these corals.

2.2.3 Scope for growth

Scope for growth, based on solely phototrophic feeding, was determined by calculating the daily amount of carbon per cm^2 of coral that is left after satisfying respiratory needs (after Anthony and Fabricius 2000). This parameter indicates whether corals can satisfy their daily respiratory needs using photosynthetic products translocated by their zooxanthellae only (i.e., are self-supporting with respect to carbon, scope for growth > 0) and how much (phototrophic) carbon would be left for growth or other processes.

To be able to calculate scope for growth, net photosynthetic rates and dark respiratory rates were converted to carbon equivalents, using the following equations:

$$P_c = P_{net} \times (12/32) \qquad [\mu mol\ C\ min^{-1}\ cm^{-2}]$$

$$R_c = R_{dark} \times (12/32) \qquad [\mu mol\ C\ min^{-1}\ cm^{-2}]$$

where P_c is net photosynthetic rate in carbon equivalents (μmol C min^{-1} cm^{-2}), P_{net} is net photosynthetic rate in oxygen equivalents (μmol O_2 min^{-1} cm^{-2}) and the factor (12/32) is the molar conversion factor to convert oxygen equivalents (O_2) to carbon equivalents (C). Analogously, R_c is dark respiratory rate carbon equivalents (μmol C min^{-1} cm^{-2}) and, R_{dark} is the dark respiratory rate in oxygen equivalents. Since we do not know the exact composition of substances that are produced during photosynthesis and that are respired during respiration, no further corrections were applied using metabolic quotients (Gattuso and Jaubert 1990).

Scope for growth was calculated using the following equation:

$$SfG = (P_c \times 10) - (R_c \times 14)$$

where *SfG* is scope for growth based on phototrophic feeding expressed in mg C h^{-1} cm^{-2}, and P_c and R_c are photosynthetic and respiratory rates expressed in mg C h^{-1} cm^{-2}. Calculations were based on a light-dark period of 10L:14D.

2.3 Coral biomass parameters

At the end of all respirometric flow cell incubations, buoyant mass, surface area and volume were once more determined for all corals. An estimate of the skeletal (bulk) density of the coral skeleton was calculated by dividing the buoyant mass (g) of the coral skeleton by the total enclosed volume (ml) of the coral skeleton (Bucher et al. 1998). Then, all corals were used for subsequent analysis of their tissue.

Corals were snap-fixed in 10% formaldehyde in 0.22 µm filtered seawater (FSW) (34 ppt) and then placed in 250-300 ml Ca^{2+}-Mg^{2+}-free artificial seawater (ASW) with ethylene diamine tetracetic acid (EDTA). This solution was prepared according to Rinkevich et al. 2005. Coral tissue was splashed off after 30 minutes ultra-sonification by using sharp water jets coming from small pipettes. Cell suspensions were collected, diluted with 0.22 µm FSW and centrifuged twice for 10 minutes at 3°C at 4000 rpm, after which total volume was determined.

Each tissue sample was homogenized using a LABOCAT X1030, after which subsamples were taken for analyses of chlorophyll, ash-free dry mass, and CN content. Chlorophyll was extracted by adding 9 ml 100% acetone to 900-1000 µl tissue homogenate and storing it at -20°C overnight. Absorbance of the extract was measured in triplicate using a Beckman Coulter DU 530 Spectrophotometer at 750, 664 and 630 nm. 90% acetone was used as a blank. The concentrations of chlorophyll A and chlorophyll C_2 were computed according to the equations given by Jeffrey and Humphrey (1975) for dinoflagellates.

A duplicate of approx. 4-7 ml tissue homogenate of each coral was dried at 103°C until constant mass and then burnt at 550°C until constant mass. Ash-free dry mass was calculated by subtracting the mass of the ashes from dry mass. Due to the small amount of tissue of the corals from the zero flow treatment, the tissue sample used for chlorophyll analysis was recovered for analysis of ash-free dry mass by evaporating the acetone at 60°C inside a fume hood and resuspending the pellet in a final volume of 1 ml 0.22 FSW (34 ppt).

CN analysis was done using an EA 1108 CHN-O from Fisons Instruments. Approximately 10 µl of wet tissue suspension (approx. 10-15 µg in dry mass) was used for each measurement, measuring each coral in triplicate. Atropine was used as a standard.

2.4 Data analysis

Normality (p>0.05) and homogeneity of variance (p>0.05) of the data were tested using Shapiro-Wilk and Levene's test in SAS 9.1. Since our data did not satisfy the assumptions for ANOVA testing, we used Kruskall Wallis as a non-parametric test to detect statistical differences between treatments.

3. Results

3.1 Growth parameters

3.1.1 Buoyant mass

All corals grew in buoyant mass during the experiment ($p<0.001$, Figure 3). The first significant differences in buoyant mass between flow regimes became apparent after 12 weeks (110 days after nubbing) ($p=0.0005$).

At the end of the growth experiment (week 42), the corals in the 0 cm s^{-1} flow treatment had a significant lower buoyant mass compared to the corals in the 10, 20 and 25 cm s^{-1} flow treatment ($p=0.0002$), while the corals in the 25 cm s^{-1} flow treatment had a significantly higher buoyant mass compared to the corals in the 0, 10, and 20 cm s^{-1} flow treatment ($p<0.0005$). No difference was detected between the corals I nthe 10 cm s^{-1} and 20 cm s^{-1} flow treatment ($p=0.82$). The corals in the 0 cm s^{-1} flow treatment appeared unhealthy, their tissue was pale and showed regression from time to time.

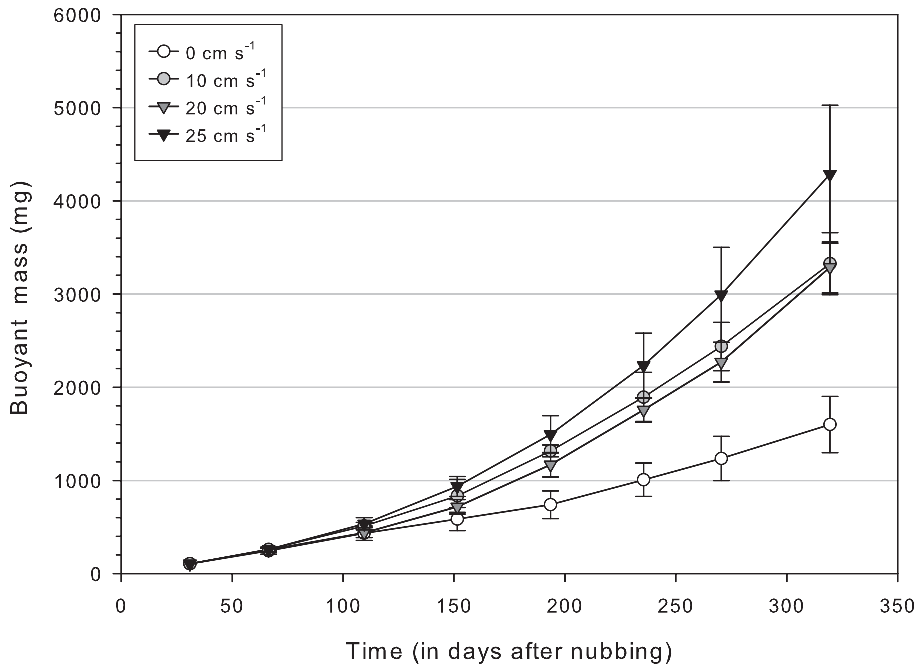

Figure 3: *The effect of flow regime on buoyant mass increase during the experimental period. Values are mean ± SD, N=10.*

3.1.2 Surface area

The increase in surface area during the experiment (Figure 4), gave a similar picture as the increase in buoyant mass (Figure 3), except that differences in surface area between treatments became apparent only at week 15 (135 days) ($p<0.0001$).

Growth in surface area was found to be not as continuous as in buoyant mass. When growing, *G. fascicularis* appears to first create a layer of tissue around the coral (in a circular fashion), in which ultimately some polyps will be formed. Thus, this type of growth occurs in “bursts”. Skeletal growth, on the other hand, is an ongoing process also occurring in the central part of the colony. Growth in surface area is also much more hindered by algal competition than growth as buoyant mass (visual observation).

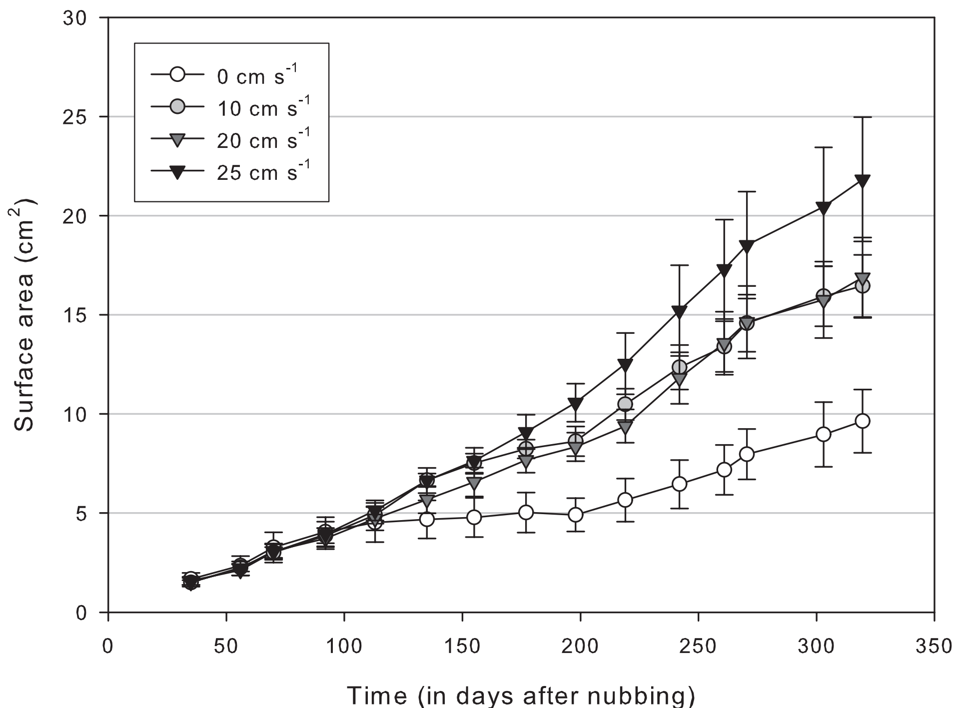

Figure 4: *The effect of flow regime on surface area increase during the experimental period. Values are mean ± SD, N=10.*

At the end of the growth experiment (week 42, 320 days), the relative differences in growth as surface area between flow treatments were similar to the relative differences

in growth as buoyant mass: 0 cm s^{-1} flow treatment had a significant lower surface area compared to the corals in the 10, 20 and 25 cm s^{-1} flow treatment (p=0.0002), while the corals in the 25 cm s^{-1} flow treatment had a significantly higher surface area compared to the corals in the 0, 10, and 20 cm s^{-1} flow treatment (p≤0.0012). No difference was detected between the corals in the 10 cm s^{-1} and 20 cm s^{-1} flow treatment (p=0.8206).

3.1.3 Polyp number

The increase in polyp number during the experiment (Figure 5) gives a slightly different picture than the increase in buoyant mass (Figure 3) and surface area (Figure 4).

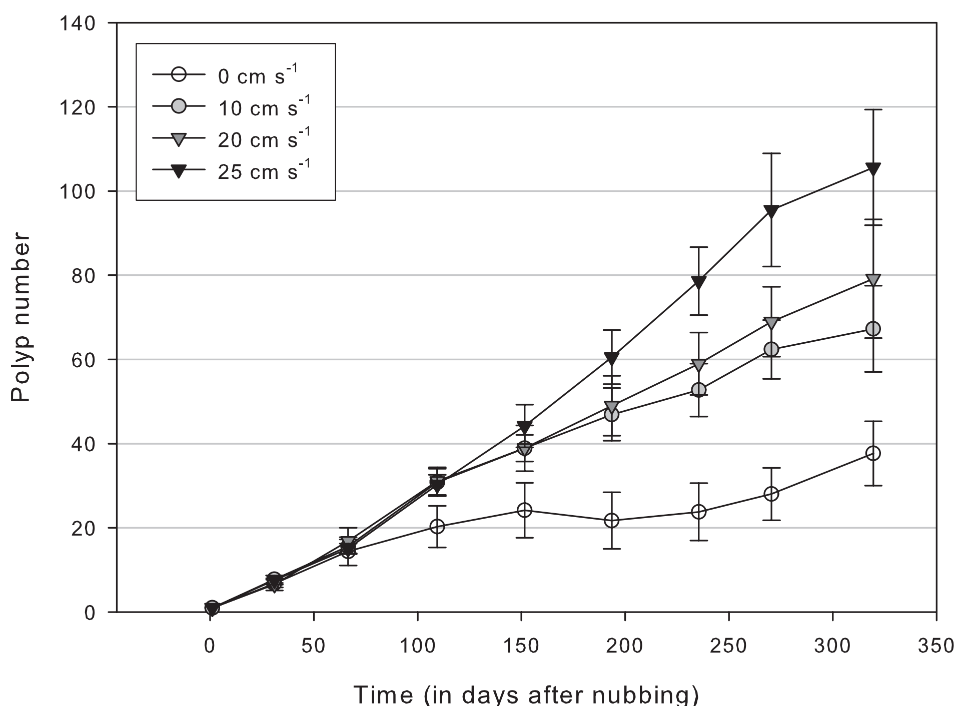

Figure 5: *The effect of flow regime on polyp number increase during the experimental period. Values are mean ± SD, N=10.*

Just as with buoyant mass, the first significant differences in polyp number between flow regimes became apparent at week 12 (110 days after nubbing) (p<0.0001). However, at the end of the growth experiment (week 42), polyp numbers had significantly increased

with each flow regime. The 0 cm s^{-1} flow treatment had a significant lower polyp number compared to the corals in the 10, 20 and 25 cm s^{-1} flow treatment ($p<0.0003$). The 10 cm s^{-1} flow treatment had a significant lower polyp number compared to the corals in the 20 and 25 cm s^{-1} flow treatment ($p <0.05$) and the 20 cm s^{-1} flow treatment had a significantly lower polyp number than the corals in the 25 cm s^{-1} flow treatment ($p <0.005$)

3.1.4 Growth kinetics

Specific growth rates were calculated per measurement interval using the buoyant mass data (Figure 6). It is seen that the specific growth rate decreases with time, implying that the growth of *G. fascicularis* is not exponential. Within measurement intervals, the growth of the 0 cm s^{-1} nubbins is consistently lower, with exception for the growth interval between week 6 and 12 where the specific growth rate of the 20 cm s^{-1} nubbins was decreased as well. The differences in specific growth rates between the corals in the 10 cm s^{-1}, 20 cm s^{-1} and 25 cm s^{-1} flow treatment are not consistent.

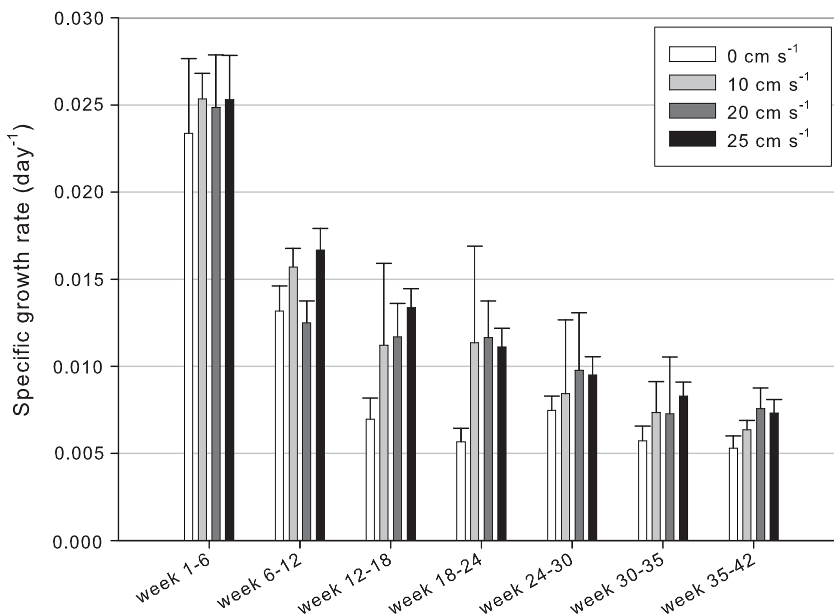

Figure 6: *Specific growth rates (day^{-1}) based on coral buoyant mass and calculated for each measurement interval. Values are mean ± SD, N=10.*

On average over the entire experimental period (week 1- week 42), specific growth rate was 0.0094 ± 0.0007 day^{-1} in the 0 cm s^{-1} flow treatment, 0.0119 ± 0.0004 day^{-1} in the 10 cm s^{-1} flow treatment, 0.0119 ± 0.0003 day^{-1} in the 20 cm s^{-1} flow treatment and 0.0128 ± 0.0006 day^{-1} in the 25 cm s^{-1} flow treatment.

3.2 Respirometric measurements

3.2.1 Net photosynthesis and dark respiration

Net photosynthesis decreased with flow speed. Significant differences were detected between 10 cm s^{-1} (11.7 ± 1.8 nmol O_2 min^{-1} cm^{-2}) and 25 cm s^{-1} (8.2 ± 0.2 nmol O_2 min^{-1} cm^{-2})(p=0.0495) and 20 cm s^{-1} (10.4 ± 1.4 nmol O_2 min^{-1} cm^{-2}) and 25 cm s^{-1} (p=0.0495) (Figure 7).

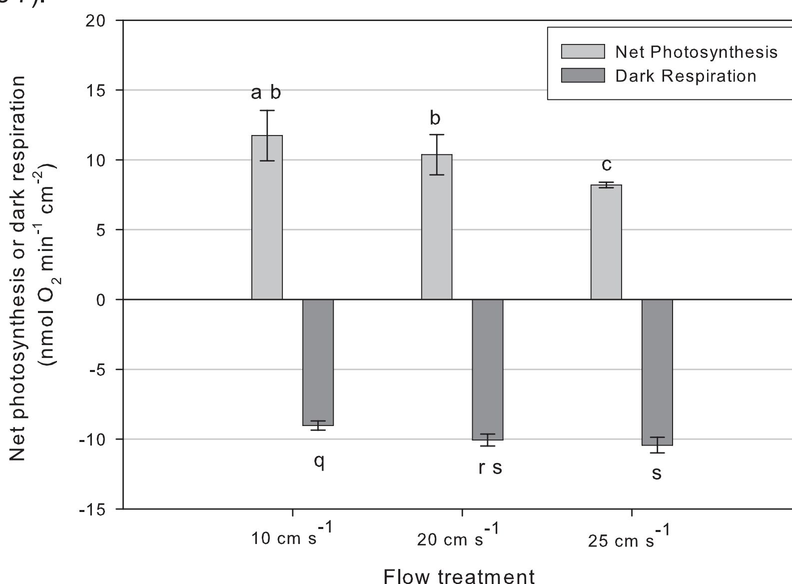

Figure 7: *The effect of water flow speed on net photosynthesis and dark respiration. Values are mean ± SD N=3. Means lacking a common superscript differ significantly (p<0.05).*

Dark respiration significantly increased between 10 cm s^{-1} (-9.0 ± 0.3 nmol O_2 min^{-1} cm^{-2}) and 20 cm s^{-1} (-10.0 ± 0.4 nmol O_2 min^{-1} cm^{-2}) (p=0.0495) and 10 cm s^{-1} and 25 cm s^{-1} (-10.4 ± 0.6 nmol O_2 min^{-1} cm^{-2}) (p=0.0495). No significant difference was detected between 20 and 25 cm s^{-1} (p=0.28) (Figure 7).

During these short-term incubation experiments, the skeletal growth rate (in day^{-1}) of the experimental corals (n=3) in each flow treatment was not significantly different (p>0.14) from the corals that remained untouched in the flow experiment (n=7). Thus, during this time period no significant effect of handling on skeletal growth rate was detected.

3.2.2 Scope for growth

Scope for growth was -0.54 ± 0.98 SD µmol C cm^{-2} day^{-1} for 10 cm s^{-1}, -2.22 ± 0.81 SD µmol C cm^{-2} day^{-1} for 20 cm s^{-1} and -3.84 ± 0.35 SD µmol C cm^{-2} day^{-1} for 25 cm s^{-1} (Figure 8). The amount of carbon per cm^2 left for other processes decreased with increasing flow rate. It was significantly lower in the 25 cm s^{-1} flow treatment compared to the 20 cm s^{-1} flow treatment (p=0.02). No significant difference was found between 10 cm s^{-1} and 20 cm s^{-1} (p=0.60) and 10 and 25 cm s^{-1} (p=0.09).

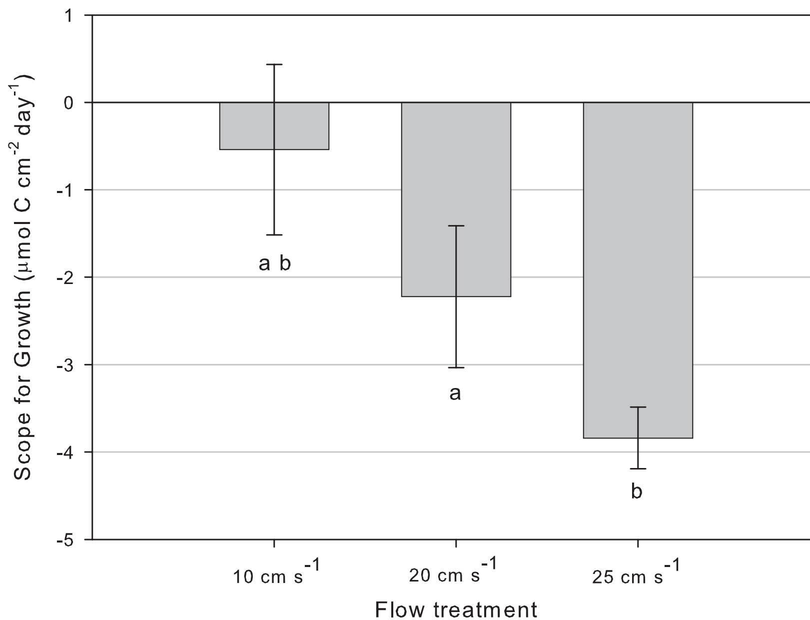

Figure 8: *Influence of flow speed on the daily amount of photosynthetic carbon left for growth (µmol C cm^{-2} day^{-1}). Values are mean ± SD N=3, 3 measurements averaged per coral. Means lacking a common superscript differ significantly (p<0.05).*

3.3 Coral biomass parameters

Coral biomass parameters were determined after the ending of the short-term incubation experiments. Since the health of corals in the 0 cm s^{-1} treatment became more and more impaired after the 42 week growth experiment, the biomass parameters measured of the corals in the 0 cm s^{-1} treatment do not necessarily reflect the status of these corals during the growth experiment. The health of the corals in the other treatments was not impaired, and therefore their biomass parameters are assumed to be representative of the status of the corals during the entire experiment.

3.3.1 Skeletal density

A proxy of skeletal density was calculated by dividing buoyant mass of each coral (g) by its volume (ml), after subtracting mass and volume of their PVC plates. This measure of skeletal density was 0.18 ± 0.03 g ml^{-1} for the corals in the 0 cm s^{-1} treatment, 0.67 ± 0.06 g ml^{-1} for 10 cm s^{-1}, 0.79 ± 0.11 g ml^{-1} for 20 cm s^{-1} and 0.75 ± 0.11 g ml^{-1} for 25 cm s^{-1}. The corals in the zero flow treatment had a significantly lower skeletal density than the others ($p<0.005$), while the skeletal density of the corals in the other flow treatments were not significantly different from each other ($p>0.08$)

3.3.2 Coral tissue parameters

Ash free dry mass of coral tissue per surface area was significantly lowest in the 0 cm s^{-1} treatment (3.6 ± 1.5 µg cm^{-2}, $p<0.008$), while it was significantly highest for the corals in the 10 cm s^{-1} treatment (8.4 ± 1.9 µg cm^{-2}, $p<0.03$). No significant difference was found between the corals in 20 cm s^{-1} (5.5 ± 1.1 µg cm^{-2}) and 25 cm s^{-1} (5.4 ± 1.3 µg cm^{-2}) ($p=0.74$) (Figure 9).

The percentage of carbon in coral tissue (41.4 ± 7.6 %, calculated as mg C per mg ash free dry mass of tissue x 100%) is not significantly different between treatments ($p>0.12$). The percentage nitrogen in coral tissue, however, showed small, but significant differences between 0 cm s^{-1} (7.41 ± 0.68) and 20 cm s^{-1} (5.99 ± 0.68) ($p=0.015$) and between 0 cm s^{-1} and 25 cm s^{-1} (6.03 ± 0.35) ($p=0.007$), with a higher percentage nitrogen in the tissue

of the 0 cm s^{-1} corals. The C:N ratio is increasing slightly with flow speed: 5.65 ± 1.23 for 0 cm s^{-1}, 6.07 ± 0.90 for 10 cm s^{-1}, 6.56 ±1.17 for 20 cm s^{-1} and 7.18 ± 1.21 for 25 cm s^{-1}. The C:N ratio of coral tissue of corals maintained at 25 cm s^{-1} was significantly higher than that of corals maintained at 0 cm s^{-1} ($p=0.022$).

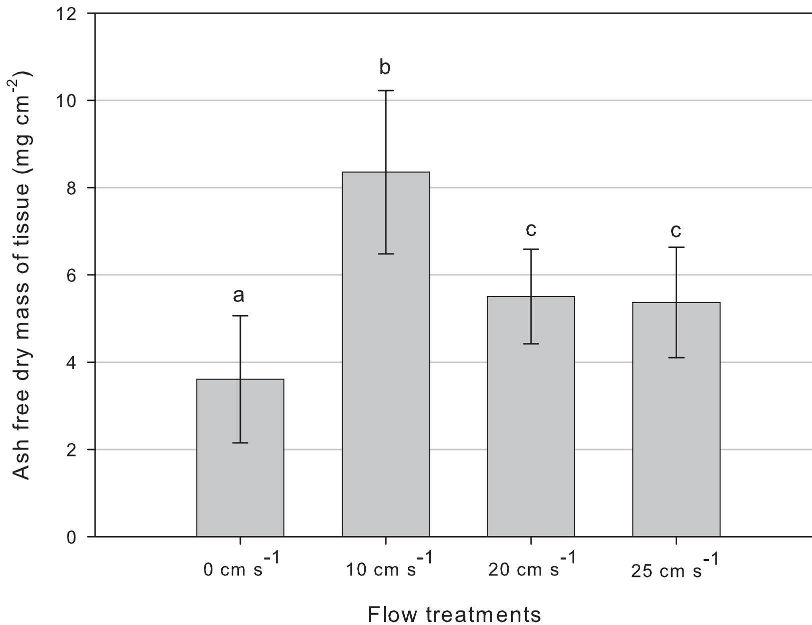

Figure 9: *The effect of flow regime on the ash free dry mass of coral tissue per surface are of coral (mg cm^{-2}). Values are mean ± SD N=10 for 0, 20 and 25 cm s^{-1}, N=5 for 10 cm s^{-1}. Means lacking a common superscript differ significantly ($p<0.05$).*

The amount of chlorophyll A (Chl A) and chlorophyll C_2 (Chl C_2) per coral surface area was significantly lower for the corals in the 0 cm s^{-1} flow treatment (1.0 ± 0.3 µg Chl A cm^{-2} and 0.6 ± 0.2 µg Chl C_2 cm^{-2} compared to the corals in the 10 cm s^{-1} flow treatment (3.8 ± 1.5 µg Chl A cm^{-2} and 2.7 ± 1.1 µg Chl C_2 cm^{-2}) and 25 cm s^{-1} flow treatment (4.1 ± 0.6 µg Chl A cm^{-2} and 2.0 ± 0.5 µg Chl C_2 cm^{-2})($p<0.005$). No significant difference was detected between 10 cm s^{-1} and 25 cm s^{-1} ($p=0.48$). The samples of the corals in the 20 cm s^{-1} were lost. Since the values for OD664 of the corals from the 0 cm s^{-1} flow treatment were often below the detection limit, the absolute values should be interpreted with caution.

4. Discussion

4.1 Water flow and growth

Skeletal growth of *G. fascicularis* increased with time in all flow treatments. Since differences in growth between treatments only started to become apparent at week 12, growth experiments using *G. fascicularis* nubbins should last at least for 12 weeks.

This study demonstrates the importance of water flow to the growth of the scleractinian coral *G. fascicularis*. Absence of flow resulted in significant lower specific growth rates. An increase in growth was found between 0 and 10 cm s^{-1}, which is in agreement with the findings of Jokiel (1978) in the range of 2-15 cm s^{-1} for *Pocillopora meandrina* and *Pocillopora damicornis*. However, no significant difference in growth was found between the 10 cm s^{-1} and 20 cm s^{-1} flow treatments, while the corals in the 25 cm s^{-1} flow treatment had a significant higher skeletal growth than the corals in the other flow treatments. The same trend was found for surface area, while polyp number was significantly increased with increasing flow. Surface area and polyp number are, however, more an expression of morphology than of clear-cut skeletal growth.

Specific growth rate of *G. fascicularis* decreased with time, as observed before by Schutter et al. (2008), and fell within the range of specific growth rates to be expected for *G. fascicularis* at an irradiance of 90 μ E m^{-2} s^{-1} (± 0.012 day^{-1}, Schutter et al. 2008). Although the differences in specific growth rates between flow treatments were not consistent between measurement intervals (Figure 6), the specific growth rate calculated over the entire experimental period showed the same trend as for the growth parameters.

4.2 Water flow and phototrophic metabolism

In the following paragraphs, the relation of net photosynthesis, dark respiration and scope for growth with specific growth rate is discussed in an attempt to explain the relationship between water flow and growth and possible relieve of mass transfer limitations. It can be questioned whether it is legitimate to use metabolic rates of corals measured at the

end of the experiment to explain differences in the growth of these corals during the experiment. First, it can be argued that each physiological measurement is just a random indication of the metabolic rate at the time of measurement. However, by measuring the metabolic rate of each coral at various times at different days this potential artifact is reduced. Second, it can be argued that measurements cannot be extrapolated due to differences in size. However, a strong linear correlation is found between metabolic rate (mg O_2 per min) and surface area (cm^2) (Adj R^2 =0.65, $p<0.01$ for photosynthesis, Adj R^2 =0.91, $p<0.01$ for respiration) over a size range of 5-50 cm^2, indicating that the measured metabolic rates are representative for a large range of coral sizes.

4.2.1 Photosynthesis

Reduction of the diffusive boundary layer thickness with increasing water flow did not result in increased net photosynthetic rates. In contrast, the net photosynthetic rate was not significantly different between 10 cm s^{-1} and 20 cm s^{-1}, and the net photosynthetic rate at 25 cm s^{-1} was even significantly lower compared to 10 cm s^{-1} and 20 cm s^{-1}. In this study, net photosynthetic rates are thus not positively correlated with growth, in contrast to Schutter et al. (2008). Obviously, photosynthates are not effectively channeled into skeletal growth and possibly allocated to different processes than skeletal growth.

Different effects of water flow on photosynthesis have been reported. Sebens et al. (2003) found no differences in the rate of net photosynthesis between 2, 5, 8 and 10 cm s^{-1} for *Agaricia tenuifolia*, while Lesser et al. (1994) found a positive effect of increasing flow on net photosynthesis of *Pocillopora damicornis* (mean flow: 0.2, 3.8 and 7.2 cm s^{-1}). The decline we found in net photosynthesis at flow rates higher than 10 cm s^{-1} cannot be verified with other data from literature, since, to the best of our knowledge, no data in literature are available for comparison. At low irradiances, such as used in this study, water flow may have a smaller effect on photosynthesis, since the need for inorganic carbon supply and/or removal of oxygen to optimize photosynthesis at low irradiance levels is not as demanding as at high irradiance levels (Nakamura et al. 2005; Finelli et al. 2006; Smith and Birkeland 2007).

Water flow did not have an effect on chlorophyll A and chlorophyll C_2 content per surface

area, which is in agreement with the findings of Stambler et al. (1991) and Lesser et al. (1994) for *Pocillopora damicornis* and of Rex et al. (1995) for *Porites cylindrica*. Apparently, irradiance has a dominant effect on photosynthesis and chlorophyll content, while water flow rates can only modulate uptake and release rates of substances needed for photosynthesis.

4.2.2 Respiration

Reduction of the diffusive boundary layer thickness with increasing water flow resulted in increased dark respiratory rates between 10 cm s^{-1} and 20 cm s^{-1}, and between 10 cm s^{-1} and 25 cm s^{-1}. Sebens et al. (2003) reported an increase in dark respiration with increasing flow speed for *Agaricia tenuifolia* between 2 and 10 cm s^{-1}. However, our data cannot be verified with data that have been collected at flow speeds above 10 cm s^{-1}, since, to the best of our knowledge, such data have not been reported.

Respiration increases the availability of metabolic CO_2 that can be used as a source of carbon for calcification (70% of DIC for calcification comes from metabolic CO_2: Furla et al. 2000) and generates energy that can be used for calcification. Therefore, respiration could potentially be limiting for growth. The decreased growth of the corals in the 0 cm s^{-1} flow treatments compared to the other flow treatments could possibly be the result of a limited supply of oxygen and consequently a reduced dark respiratory rate and reduced availability of metabolic CO_2. Nevertheless, the difference in growth between 20 cm s^{-1} and 25 cm s^{-1} cannot be explained with dark respiratory rates, since this difference was not significantly different. Respiratory rates during the day (light respiration) will probably be independent of flow-related mass transfer, since oxygen used for respiration is not limiting due to photosynthetic production of oxygen in the light.

4.2.3 Scope for Growth

Scope for growth based on phototrophic feeding (i.e., daily amount of photosynthetic carbon that is left for other processes after satisfying respiratory needs) was negatively correlated with growth rate, indicating that phototrophic carbon was not instrumental in supporting higher skeletal growth rates.

Since the average net photosynthetic rate in this study was 50% lower than what we would expect based on previous photosynthesis-irradiance curves of *G. fascicularis* measured under similar conditions (Schutter et al. 2008), and average respiratory rate was threefold higher, absolute values should be interpreted with caution. Differences between respirometric values could possibly be due to the short-term nature of the measurement of a photosynthesis-irradiance curve and the influence of the growth irradiance on the result. A photosynthesis-irradiance curve provides information about the potential to adapt to another irradiance within a short time, while in the long run the photosynthetic rate at a certain irradiance might be different due to long term adaptations of the coral's physiology. Nevertheless, these data do provide a qualitative indication of the effect of flow on photosynthesis, respiration and scope for growth.

While the corals in the 10 cm s^{-1} flow treatment had the highest scope for growth, this did not seem instrumental in supporting higher skeletal growth rates. It might be possible that this carbon was not allocated to skeletal growth but to tissue growth, since ash free dry mass of the coral tissue was significantly higher. Since the average C:N ratio of tissue of the 10 cm s^{-1} corals was not significantly different from the other flow treatments, no difference in the composition of the tissue is expected. This excludes the possibility that the 10 cm s^{-1} corals had stored more (carbon rich) storage compounds (Glynn et al. 1985; Harland 1992; Anthony et al. 2002), which would occur if the amount of translocated carbon-rich photosynthetic products exceeds that what is necessary to keep pace with skeletal growth (Anthony et al. 2002). The tissue mass thus seems normal. The average C:N ratio of coral tissue (6.37 ± 1.26) found in this study (with exception of 0 cm s^{-1} corals) is in line with values reported in literature for *Montastrea annularis* (7.5, Szmant and Gassman 1990) and *Pocillopora damicornis* (8-10, Lesser et al. 1994) and suggests total nutrient sufficiency.

4.3 Water flow and energy allocation

As evident from the previous, the increased growth at 25 cm s^{-1} compared to the growth at 10 and 20 cm s^{-1} differences in growth between flow treatments cannot be explained

by the availability of photosynthetic carbon. Alternative potential explanations are that: 1) corals at high flow have better access to other sources of carbon (e.g., *Artemia*, DOC) resulting in an increase in scope for growth (based on both sources of carbon) with water flow speed (Atkinson and Bilger 1992), and/or 2) corals at high flow have increased supply of different requirements for calcification (e.g., HCO_3^-), and/or 3) different allocation processes played a role energy was allocated among different biological functions. e.g., energy trade-off between skeletal growth, tissue growth and competition.

Tissue mass of the corals in the 20 cm s^{-1} and 25 cm s^{-1} flow treatment was significantly lower compared to 10 cm s^{-1}. The decreased availability of photosynthetic carbon with flow does not seem to explain the decreased tissue mass, since a similar amount of carbon was allocated to both the 20 cm s^{-1} and 25 cm s^{-1} corals. Moreover, despite a lower availability of photosynthetic carbon, a significant amount of carbon was allocated to skeletal growth in the case of the 25 cm s^{-1} corals. The corals in the 25 cm s^{-1} treatment must therefore either have had more access to other sources of carbon, or the available carbon was allocated to different processes. Since the corals in the 25 cm s^{-1} flow treatment suffered the least competition with algae, it is possible that they were able to allocate more energy to skeletal growth than to defense or repair mechanisms against competing algae. The inconsistent differences in specific growth rates between growth intervals among the corals in the 10 cm s^{-1}, 20 cm s^{-1} and 25 cm s^{-1} treatment confirm this differential energy allocation as well. Although the corals in the lower flow treatments (10 cm s^{-1} and 20 cm s^{-1}) were be able to express a similar growth potential as the corals in the 25 cm s^{-1} flow treatment on some occasions, they did not express it as regularly. Apparently, corals growing at higher water flow rates are less often disturbed in growth by competing algae and will grow more often with higher specific growth rate. Therefore, a combination of the individual growth potential under the given environmental conditions and the individual presence of disturbances will determine the resulting specific growth rate. Although differential energy allocation might be a possible explanation for our contradictory findings (increased growth but decreasing phototrophic metabolism), more research is needed to confirm this.

It is expected that phototrophic feeding and the modulation of photosynthesis by water flow

becomes more important at high irradiance. Further research describing the interaction between water flow and irradiance level is in progress.

4.4 Water flow and skeletal density

Skeletal density was significantly lower in the absence of flow. This can be considered as abnormal, since the corals in the 0 cm s^{-1} flow treatment were unhealthy at the time of sampling. They had very little pigmentation and a lower tissue biomass, which occurs in bleached corals (Szmant and Gassman 1989).
G. fascicularis developed a denser skeleton at higher flow regimes. However, the absence of differences in skeletal density with increasing flow rate (10, 20, 25 cm s^{-1}) suggests that they do not further strengthen their skeleton to withstand physical damage from hydrodynamic energy (Schuhmacher and Plewka 1981; Bucher et al. 1998). Possibly, it is not necessary for a massive mound-shaped coral like *G. fascicularis* to increase skeletal strength, since its growth form does not obstruct the water flow as much as a branching coral would.

Acknowledgements

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Chapter 3

The effect of irradiance on long-term skeletal growth and net photosynthesis in *Galaxea fascicularis* under four light conditions

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This chapter is an extended version of Schutter et al. (2008).

Abstract

The relation between irradiance, skeletal growth and net photosynthesis was studied for the scleractinian coral *Galaxea fascicularis* to provide experimental evidence for mediation of light-enhanced calcification through photosynthesis. The hypothesis was tested that skeletal growth and photosynthesis are linearly correlated.

A long-term experiment was performed in a closed-circuit aquarium system, in which four series of nine nubbins (single polyp clones of a coral colony) of *G. fascicularis* were exposed to four light treatments (10L:14D): 144 W T8 fluorescent lighting providing an irradiance of 68 μE m^{-2} s^{-1} and 70, 250 and 400 W Metal Halide lighting providing an irradiance of 38 μE m^{-2} s^{-1}, 166 μE m^{-2} s^{-1} and 410 μE m^{-2} s^{-1}, respectively. Growth of these nubbins was measured as buoyant mass at different time intervals in a 294 day experiment. A light-saturation curve for photosynthesis was measured in a respirometric flow cell using a 54 week *G. fascicularis* colony grown at 60 μE m^{-2} s^{-1}.

No saturation of net photosynthesis of *G. fascicularis* was found at the irradiances tested. The specific growth rate (μ, in day^{-1}) of the coral nubbins increased with irradiance. Whereas irradiance varied 11-fold (38 to 410 μE m^{-2} s^{-1}), buoyant mass (increase after 294 days) increased 5.7 times (2243 to 12374 mg), specific growth rate (1-294 days) increased 1.6 times (0.0103 to 0.0161 day^{-1}), while net photosynthetic rate increased 8.9 times (0.009 μmol O_2 min^{-1} cm^{-2} to 0.077 μmol O_2 min^{-1} cm^{-2}). The increase of specific growth rate with irradiance was less than expected based on the increase in net photosynthetic rate with irradiance. This discrepancy between potential energy produced in photosynthesis and energy used for skeletal growth indicates that skeletal growth is not limited by photosynthetic potential at high irradiance levels.

Key words: *Scleractinian coral,* Galaxea fascicularis*, irradiance, skeletal growth, buoyant mass, photosynthesis*

3

1 Introduction

Light is one of the most important abiotic factors influencing the growth of scleractinian corals. Scleractinian corals live in symbiosis with unicellular algae, known as zooxanthellae, that reside in their endodermal tissue layers. In the light, zooxanthellae perform photosynthesis, during which process they produce oxygen and organic compounds. When their own respiratory needs are satisfied, zooxanthellae translocate the excess photosynthetic products to the coral host (Muscatine and Cernichiari 1969; Muscatine et al.1981). Zooxanthellae can thus provide a considerable part of the energy needed for coral growth.

Growth of scleractinian corals can be divided in two components: first, skeletal growth due to the deposition of an external skeleton of calcium carbonate aided by the synthesis of an organic matrix in a process called calcification, and second, tissue growth. According to the light-enhanced calcification theory (see Gattuso et al. 1999 and Allemand et al. 1998b for review), the symbiosis with zooxanthellae is aiding to the process of skeletal growth. According to this theory, calcification of the coral host is enhanced by photosynthesis of zooxanthellae (Goreau and Goreau 1959; Pearse and Muscatine 1971; Allemand et al. 2004). Indeed, on average, calcification in light is found to be around three times higher than calcification in darkness (review by Gattuso 1999). Although photosynthesis and calcification are spatially separated processes (photosynthesis occurs in the oral tissue layer and calcification in the aboral tissue layer), they do share a common pool of inorganic carbon inside the coelenteron of the coral host, accounting for the interaction between these two processes. The exact mechanisms of the enhancement of calcification by photosynthesis are still a matter of debate (Gatusso et al. 1999; Furla et al. 2000). Some of the proposed mechanisms include that: 1) photosynthesis provides energy for the energy-demanding processes associated with calcification, such as calcium transport and organic matrix synthesis (Wainwright 1963; Chalker and Taylor 1975), and 2) photosynthesis raises intracellular pH and intracellular saturation state of calcium carbonate, thereby favoring the precipitation of calcium carbonate (Goreau and Goreau 1959; Allemand et al. 1998).

The relation between light and photosynthesis can be quantitatively described using light-dependent models (Chalker 1981), resulting in photosynthesis-irradiance curves. At low irradiance, the rate of photosynthesis is nearly directly proportional to irradiance. At higher irradiance, the rate of photosynthesis rapidly approaches a horizontal asymptote, which is the point where saturation of photosynthesis is reached (the maximum gross photosynthetic rate, P^g_{max}). Calcification can be described using the same light-dependent models (Chalker 1981).

Since scientists started to study coral calcification some 50 years ago, several authors have found a positive correlation between light and calcification, either in the field (Bosscher and Meesters 1993) or through experimental work (e.g. Goreau 1959; Marubini 2001; Reynaud-Vaganay et al. 2001; Reynaud et al. 2004; Schlacher et al. 2007).

However, none of these authors coupled their growth data to a photosynthesis-irradiance curve. Although it has been demonstrated by eg. Al-Horani et al. (2005) that higher rates of skeletal growth in *G. fascicularis* are supported by higher rates of photosynthesis and respiration in the adjacent polyp parts (Al Horani et al. 2005), it cannot be derived from either of these studies to what extent an increase in photosynthesis leads to a proportional increase in skeletal growth.

To the best of our knowledge, a study describing the relation between light, photosynthesis and skeletal growth – i.e. the result of calcification - of individual corals followed in time under controlled conditions is still lacking. We examined this relation by measuring the growth of the scleractinian coral *G. fascicularis* under four different irradiance levels in a closed-circuit aquarium system and comparing the results to a photosynthesis-irradiance curve of this species. In this study, the hypothesis was tested that skeletal growth and photosynthesis are linearly correlated.

2 Materials and Methods

Thirty-six (36) coral nubbins (single polyp clones) of *G. fascicularis* were created of colonies that were grown at a light intensity of ~ 60 μE m^{-2} s^{-1} (70W HQI) in a closed-circuit coral aquaculture system "Quarantine system QU4" of Burgers Ocean, Arnhem, The Netherlands. Each coral nubbin was fixed to a 5x5cm perforated PVC plate using Reef Construct (Aquamedic). Nine plates of coral nubbins were fixed to one single square plate and assigned to each of the following four experimental treatments: 70 W Metal Halide (MH) lighting (BLV Hit-Lite, HIT-DE, 10.000K), 144 W fluorescent T8 lighting (2x Philips TLD 36W/950 (5300 K), 2x Osram L 36W/67 (blue)), 250 W MH lighting and 400 W MH lighting. A light dark cycle of 10L:14D was applied. As a result of working inside a public aquarium such as Burgers Ocean, we were constrained to incorporate our experiments into existing systems, which limited our ability to standardize the experimental setup. To standardize the light regimes, the average irradiance level was determined within each experimental treatment by measuring irradiance (or photosynthetic photon flux density) at different locations under the light source. Irradiance was measured using a Li-Cor 192SA quantum underwater sensor, which measures light in the photosynthetic active region (PAR, 400-700nm). The metal halide light sources had a quite variable light distribution compared to the T8 light source. Using these light distribution patterns, the average irradiance experienced by the coral nubbins was calculated for each treatment at the start of the experiment: 38 (range: 35-45) μE m^{-2} s^{-1} for 70W MH treatment, 68 (range: 65-70) μE m^{-2} s^{-1} for 144W T8 treatment, 166 (range: 125-200) μE m^{-2} s^{-1} for 250W MH treatment and 410 (range: 300-500) μE m^{-2} s^{-1} for 400W MH treatment. Irradiance levels were measured at different times during the experiment and were found to decrease in time (at most, a 17% decrease in 274 days).

Each PVC plate containing 9 coral nubbins was placed randomly in culture system QU4, directly under the middle of each of the light sources. Flow direction inside this 1000 litre culture system was changed every 2.5 minutes. Since several other coral species were present in this coral culture system which likely influenced local flow regimes, water flow rates were measured locally under each light source to obtain the average flow rates ex-

perienced by our experimental corals. Each experimental treatment received respectively 5 ± 0 cm s^{-1}, 5 ± 2 cm s^{-1}, 15 ± 3 cm s^{-1}, 6 ± 0 cm s^{-1} as measured using a SENSA-RC2 electromagnetic current meter (Aquadata).

Culture system QU4 is a 4000 l system consisting of two 1000 l aquaria and two 1000 l sumps. The circulation system cycles 18 m^3 h^{-1} and the system is connected to a trickle tower, a Schuran Jet Stream 2 Ca^{2+} reactor, and a Schuran Aquafloater AQ250 protein skimmer.

Seawater was made up from Tropical Marine salt (Zoomix). Temperature was maintained at 26°C and salinity at 34 ppt. The system was fed 7 days a week using *Artemia* nauplii (Salt Lake) that were hatched on site and subsequently enriched using Rich Advanced feed for 24 hours. Since hatching efficiency is not constant, the amount of *Artemia* fed each day is estimated to vary between 4-8 *Artemia*/ml. Water quality parameters were measured at regular intervals.

2.1 Growth parameters

To measure growth, the buoyant mass of the coral nubbins was measured four times during a 294 day period, at t=0 , t=111, t=179 and t=294 days. These intervals were the result of practical circumstances prevailing at the facility where the experiments were carried out (e.g., a public aquarium). In spite of their irregularity, both the frequency of measuring points and the covered time range give sufficient security that the data enable to test the hypothesis. Time is expressed as days after preparation of the nubbins.

Buoyant mass is a good method to determine of skeletal growth, since coral tissue has a density which is similar to that of seawater and therefore does not contribute significantly to the buoyant mass. Tissue only comprises 1% of the total buoyant mass when tissue does not penetrate deep into the skeleton (Davies 1989). It should be noted that buoyant mass is not a good approximation of skeletal mass, since the net upward buoyancy force (i.e. the weight of seawater displaced by the coral) will result in measuring only a fraction of the absolute mass (i.e. a fractional mass). To convert buoyant mass into skeletal mass, one needs to know the density of the seawater and the volume of the coral. Moroever,

one needs to assume a constant density of the coral skeleton during the experiment. However, for the purpose of this study, knowledge of the magnitude of buoyant mass was sufficient to calculate growth rates.

Buoyant mass was measured in the laboratory by suspending each coral (plus PVC plate) on a hook in a defined volume of seawater at a constant depth. Seawater was maintained at 26°C and 34 ppt salinity. The hook was attached to an underweighing analytical balance (Kern&Sohn D-72458 Albstadt, type 870-13) using a thin nylon string (Osinga et al. 1999). Buoyant mass of each coral was measured and the average of three measurements was taken. The initial mass of the nubbins before their attachment to their PVC-plate at t=0 was estimated by weighing 5 similar-sized nubbins of a *G. fascicularis* colony on a weighing glass and taking the average. Using this parameter, it was possible to estimate the mass of the PVC plate and the amount of Reef Construct that was used to attach each coral to its plate. All our buoyant masses were corrected for this mass in order to obtain the buoyant mass of the coral colony itself. This mass was used as parameter for data analysis.

3

The growth data of buoyant mass were also used to calculate specific growth rate (μ) using the formula:

$$\mu = (\ln BW_n - \ln BW_{n-1})/\Delta t \qquad [day^{-1}]$$

where μ is the specific growth rate (day^{-1}), BW_n is buoyant mass at the end of a growth interval, BW_{n-1} is buoyant mass at the start of a growth interval and Δt is time between measurements of buoyant mass in this growth interval.

2.2 Photosynthesis-irradiance curve

A photosynthesis-irradiance curve was measured for a *G. fascicularis* colony that was grown under a 250 W Metal Halide lamp at an irradiance of ca 60 $\mu E\ m^{-2}\ s^{-1}$. Since Goiran et al. (1996) already measured a photosynthesis-irradiance curve for *G. fascicularis* , it was repeated only once to verify its applicability for this study. Net photosynthetic pro-

duction of oxygen was measured by means of intermittent flow respirometry in a 3500 cm^3 respirometric flow cell at irradiances ranging from 40 to 500 μE m^{-2} s^{-1} using irradiance intervals of ca 60 μE m^{-2} s^{-1}. It was not possible to reach irradiances higher than 500 μE m^{-2} s^{-1} using our setup. However, the range of irradiances used corresponds to the irradiances applied in the growth experiment. Respiratory consumption of oxygen was measured in the dark. Lighting was provided by a T5 lighting system (ATI) containing eight 24W Aquablue Spezial bulbs. Irradiance was measured using a Li-Cor 192SA quantum underwater sensor. A flow speed of ± 10 cm s^{-1} was applied to ensure adequate mixing and to simulate the situation in the aquarium environment (5-15 cm s^{-1}). At each irradiance, the increase in oxygen concentration was measured every 10 seconds using a luminescent oxygen probe (Hach) until a difference in concentration was detected of ± 1 mg O_2 l^{-1}. After each measurement the flowcell was flushed with “fresh” seawater from the Quarantine tank to return the oxygen concentration to the initial value before the start of the experiment and to remove possible accumulated waste products. Temperature inside the respirometric flowcell was maintained at 26 ±0.5 °C and salinity at 34±0.1 ppt. Surface area and polyp number of the coral were determined in order to normalize the respirometric data. urface area was measured as projected surface area. Pictures were taken perpendicular to the coral directly inside the aquarium system using a Nikon Coolpix S1 5.1 mp digital camera in a Nikon WP-CP5 underwater housing. Surface area was determined by image analysis using ImageJ (1.37v) by tracing the live part of the coral colony. Since tentacle extension is variable over time, no tentacles that were extending beyond the skeleton were traced for surface area. Polyp number was counted visually. Only live polyps were counted. Newly formed polyps were only counted once they started projecting from the basal skeletal plate. The volume of the coral was determined using the water displacement technique in order to correct flowcell volume for the space taken in by the coral.

Photosynthetic rates at each irradiance were estimated by regressing oxygen concentration against time. Net photosynthetic rates were calculated according to the following equation:

$P_{net} = ((V_{cell}-V_{coral}) \times slope / S$ [μmol O_2 min^{-1} cm^{-2}]

Where P_{net} is the rate of net photosynthesis (μmol O_2 min^{-1} cm^{-2}); V_{cell} is volume of respirometric flowcell (l); V_{coral} is volume of coral; slope is regression coefficient of dissolved oxygen against time (μmol O_2 l^{-1} min^{-1}), and S is surface area of coral (cm^2).
The P/I curve was fitted according to the model of Barnes and Chalker (1990) using Sigmaplot 8.0.

$P_{net} = R_{dark} + P^g_{max} \times \tanh(I/I_k)$ [μmol O_2 min^{-1} cm^{-2}]

where P_{net} is net photosynthesis as measured during respirometry in light and R_{dark} is rate of respiration as measured during respirometry in darkness. P^g_{max} is the maximum gross photosynthetic rate (defined as maximum net photosynthetic rate (P^n_{max}) minus dark respiration (R)), tanh is the hyperbolic tangent, I is irradiance and I_k is sub-saturation irradiance (i.e. irradiance at which the initial linear slope of the curve intercepts the horizontal asymptote).

2.3 Data analysis

Normality (p>0.05) and homogeneity of variance (p>0.05) of the data were tested using Shapiro-Wilk and Levene's test in SAS 9.1. Since our data did not satisfy the assumptions for ANOVA testing, we used Kruskall Wallis as a non-parametric test to detect statistical differences between treatments.

3 Results

3.1 Culture system parameters

During the experiment (22/03/2005 till 03/07/2005) the alkalinity in the system was 3.23 ± 0.54 S.D. mEq l^{-1}, calcium concentration 393.75 ± 14.36 S.D. mg l^{-1}, magnesium concentration 1290 ± 51.29 S.D. mg l^{-1}, nitrate concentration 0.19 ± 0.08 S.D. mg NO_3^--N l^{-1}, nitrite concentration 0.014 ± 0.002 S.D. mg NO_2^--N l^{-1} and phosphate concentration 0.015 ± 0.022 S.D. mg PO_4^{3-} l^{-1}.

3.2 Growth parameters

3.2.1 Buoyant mass

All corals grew during the experiment. The buoyant mass of the corals (Figure 1) increased significantly in time in each treatment ($p<0.001$).

An increase in growth as buoyant mass with increasing irradiance was observed: at day 111 and day 179, the corals in the two highest light treatments (166 µE m^{-2} s^{-1} and 410 µE m^{-2} s^{-1}) had a significant higher calculated buoyant mass ($p<0.01$) compared to corals in the two lowest light treatments (38 µE m^{-2} s^{-1} and 68 µE m^{-2} s^{-1}). At Day 294, the corals in the highest light treatment (410 µE m^{-2} s^{-1}) had a significantly higher calculated buoyant mass compared to the 166 µE m^{-2} s^{-1} treatment ($p<0.01$). On its turn, the corals in the 166 µE m^{-2} s^{-1} treatment had a significantly higher calculated buoyant mass ($p<0.001$) compared to the corals in the two lowest light treatments (38 µE m^{-2} s^{-1} and 68 µE m^{-2} s^{-1}).

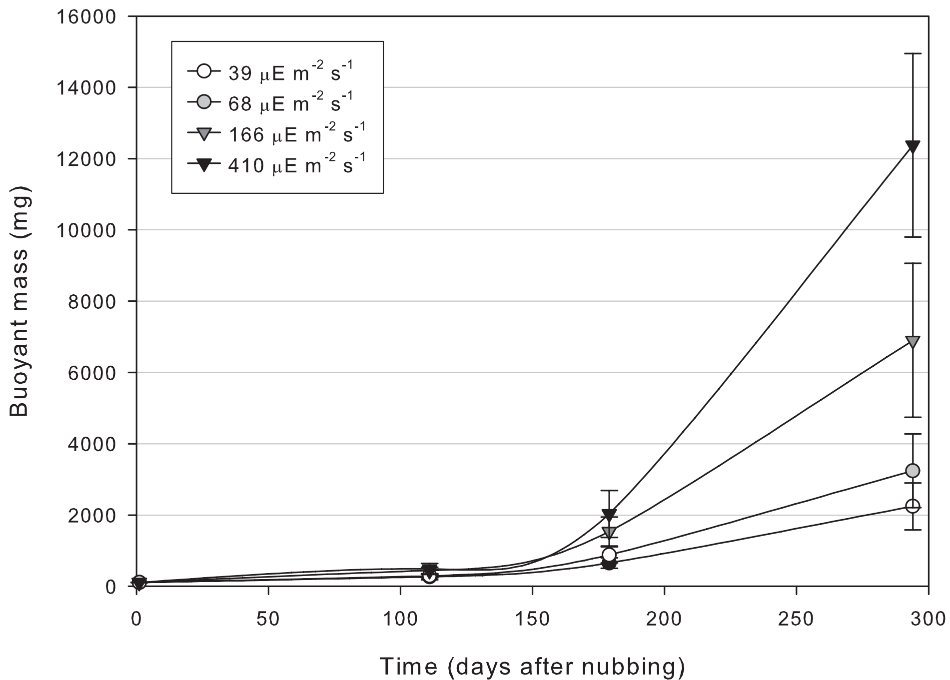

Figure 1: *Effect of irradiance on the calculated buoyant mass of* G. fascicularis *colonies. Values are mean ± stdev, N=9. Error bars indicate standard deviations.*

Differences between treatments became more pronounced during the course of the experiment. Whereas irradiance level varied 11-fold (38 to 410 µE m^{-2} s^{-1}), the average buoyant mass at Day 111 of the corals grown in the highest light treatment (410 µE m^{-2} s^{-1}) compared to the corals grown in the lowest light treatment (38 µE m^{-2} s^{-1}) was only 1.8 times increased (265 to 484 mg). At Day 179, this difference had increased to 3.1 times (652 to 2030 mg), and to 5.5 times at Day 294 (2243 to 12374 mg).

3.2.2 Specific growth rate

The specific growth rate (*µ*) of coral colonies grown under different light conditions (38 µE m^{-2} s^{-1}, 68 µE m^{-2} s^{-1}, 166 µE m^{-2} s^{-1} and 410 µE m^{-2} s^{-1}) was calculated using the calculated buoyant mass of the corals at different time intervals (1-111 days, 111-179 days, 179-294 days and 1-294 days), see Table 1.

Table 1: *Specific growth rates (μ in day^{-1}) calculated using the calculated buoyant mass of the corals are given for growth interval 1 (1-111 days), interval 2 (111-179 days), interval 3 (179-294 days) and the entire 294 days for each light condition. Means ± standard deviation are given.*

	interval 1		**interval 2**		**interval 3**		**1-294 days**	
	mean	*S.D.*	mean	*S.D.*	mean	*S.D.*	mean	*S.D.*
38 μE m^{-2} s^{-1}	0.0078	*0.0033*	0.0137	*0.0055*	0.0106	*0.0014*	0.0103	*0.0011*
68 μE m^{-2} s^{-1}	0.0090	*0.0016*	0.0161	*0.0014*	0.0112	*0.0009*	0.0115	*0.0010*
166 μE m^{-2} s^{-1}	0.0127	*0.0022*	0.0181	*0.0013*	0.0130	*0.0011*	0.0141	*0.0011*
410 μE m^{-2} s^{-1}	0.0134	*0.0028*	0.0211	*0.0007*	0.0159	*0.0011*	0.0161	*0.0007*

It is found that the specific growth rate was not constant during the experiment. In the second growth interval (111-179 days), the specific growth rates were significantly higher compared to those in the first time interval ($p<0.01$). In the third growth interval (179-294), the specific growth rates had decreased ($p<0.001$) compared to the second growth interval, except for the 38 μE m^{-2} s^{-1} treatment ($p=0.3084$). Apparently, our corals did not follow first order kinetics.

In most cases, higher irradiance supported a higher specific growth rate. When calculated over the entire 294 days time interval (Figure 2), again, the specific growth rate significantly increased with irradiance in each light treatment ($p<0.01$) except for the difference between the 38 μE m^{-2} s^{-1} and 68 μE m^{-2} s^{-1} light treatment, which was not significant ($p=0.08$).

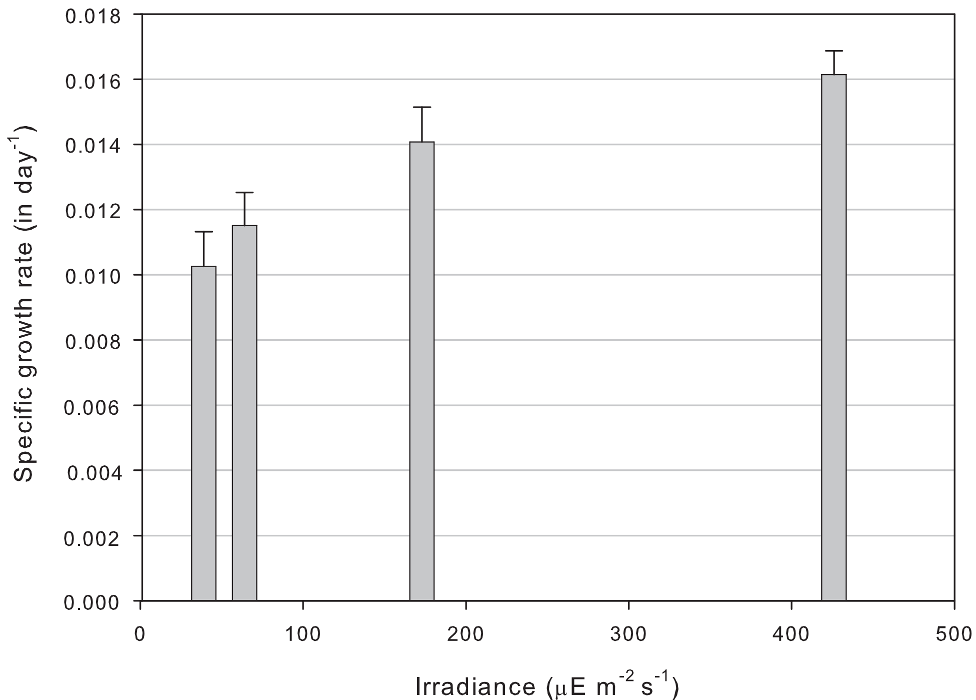

Figure 2: *Specific growth rate of* G. fascicularis *colonies grown under different light conditions calculated over the total growth period (1-294 days) and plotted against irradiance. Values are mean ± stdev, N=9.*

3.3 Photosynthesis-irradiance: comparison with specific growth rate

The relationship between photosynthetic rate and irradiance was determined for a 54 week old *G. fascicularis* colony that was grown at 60 µE m^{-2} s^{-1} receiving 144 W MH lighting (Figure 3, black dots) and compared with the effect of irradiance on the specific growth rate (from 1 to 294 days) (Figure 3, bar graph).

The photosynthesis-irradiance curve was fitted according to the model of Barnes and Chalker (1990) (Figure 3, line graph) and was found to be similar to the one measured by Goiran et al. (1996), verifying our result and its applicability for this study. Although our data did not allow a legitimate estimation of P^g_{max}, we can assert and confirm from the photosynthesis-irradiance curve measured by Goiran et al. (1996) that the irradiance

experienced by the coral nubbins in the highest light treatment in the long-term growth experiment was close to saturation.

Both specific growth rate and net photosynthesis increase with irradiance. As light varied 11-fold (38 to 410 μE m^{-2} s^{-1}), specific growth rate increased 1.6 times (0.0103 to 0.0161 day^{-1}) while net photosynthetic rate increased 8.9 times (0.009 μmol O_2 min^{-1} cm^{-2} to 0.077 μmol O_2 min^{-1} cm^{-2}). Specific growth rate does not increase proportionally with net photosynthetic rate.

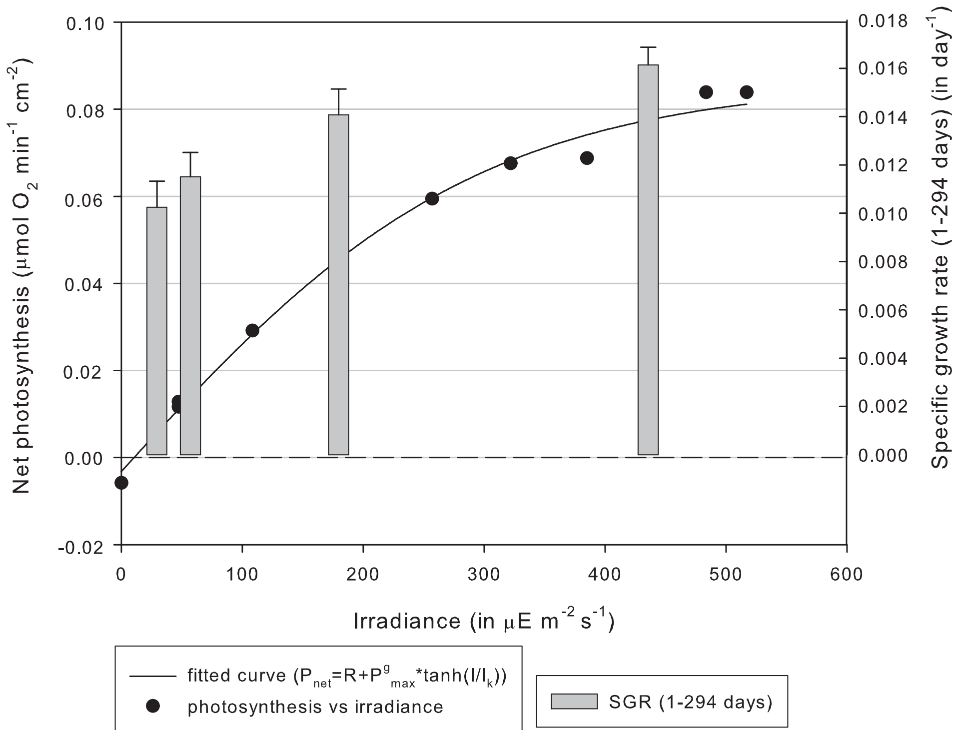

Figure 3: *Effect of irradiance on net photosynthesis in μmol O_2 min^{-1} cm^{-2} (plotted on left axis) and specific growth rate calculated from day 1 to 294 in day^{-1} (plotted on right axis). The light-saturation curve was fitted according to the model of Barnes and Chalker (1990) using Sigmaplot 8.0. Values of specific growth rate are mean ± stdev, N=9.*

4 Discussion

4.1 Growth and irradiance

Skeletal growth of *G. fascicularis* increased with increasing irradiance, which is in agreement to the positive correlations of calcification with light found by Marubini et al. (2001) for the stony coral *Porites compressa*, by Reynaud-vaganay et al. (2001) for *Stylophora pistillata* and *Acropora* sp., by Reynaud et al. (2004) for the stony coral *Acropora verweyi*, and by Schlacher et al. (2007) for *Acropora solitaryensis*.

The specific growth rate of *G. fascicularis* also increased with increasing irradiance. Inherent to the assumption of exponential growth, the relative increase in specific growth rate is less than the increase in buoyant mass over a 294 day period. However, for coral breeding in captivity, small differences in specific growth rate can result in large differences in buoyant mass increase over long time intervals.

Growth of *G. fascicularis* in this experiment did not follow first order kinetics, since specific growth rates differed between growth intervals. The first growth interval is biased because of a lag phase in growth due to regeneration after nubbing (Meesters et al. 1994). However, when comparing the specific growth rates in the second and third growth interval, it is notable that specific growth rates decrease in time. The same trend was found for *G. fascicularis* in another long-term study (M. Schutter, unpublished results). Although the exponential growth model is thus not applicable to the growth of *G. fascicularis* , it remains a proper tool to evaluate differences in growth.

4.2 Growth and photosynthesis

The relationship between net photosynthetic rate and irradiance could be approximated by a hyperbolic tangent function. The Irradiance experienced by the coral nubbins in the highest light treatment in the long-term growth experiment was close to saturation.

When comparing specific growth rates (from 1 to 294 days) in the different light treatments with the photosynthesis-irradiance curve, it was observed that net photosynthesis

increases relatively faster with irradiance than specific growth rate. In other words, specific growth rates did not increase as much as expected from the increase in net photosynthetic rate. Plotting the specific growth rate against net photosynthetic rate (Figure 4), shows that their relationship is not linear but levels off with increasing net photosynthetic rate.

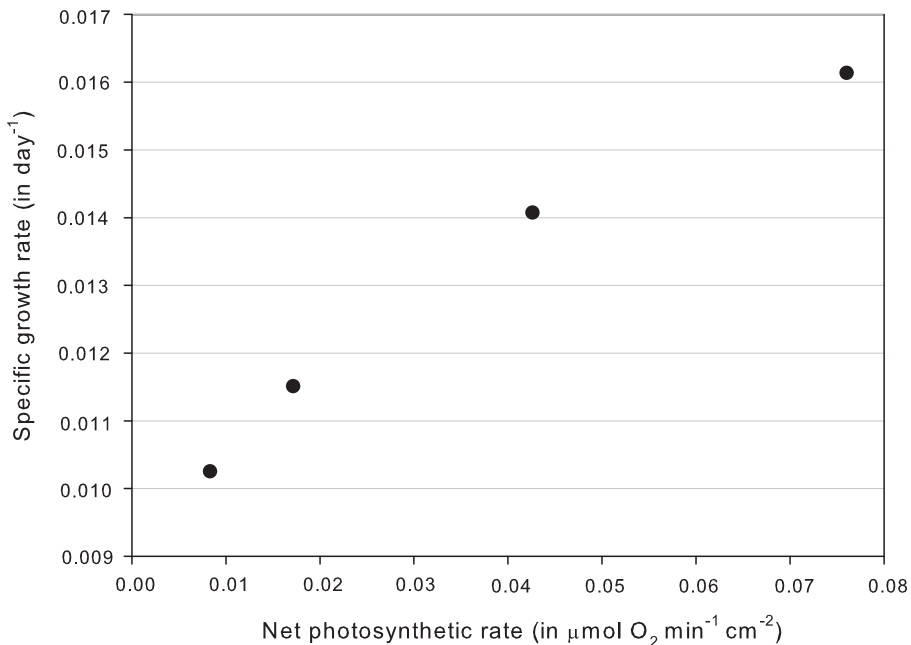

Figure 4: *Specific growth rate plotted against net photosynthetic rate, which was calculated at the same irradiances using the equation of the photosynthesis-irradiance curve.*

It is possible, that the coral's heterotrophic metabolism obscures the relation between its phototrophic metabolism and its specific growth rate in this long-term experiment. This line of thinking would suggest that the major source of energy and/or building blocks for skeletal growth is heterotrophic feeding, while phototrophic feeding is only a minor source. However, the potential energy produced in photosynthesis should not be underestimated. Part of the photosynthetically produced oxygen and photosynthates are instantly used by the coral and its zooxanthellae in the process of light respiration, generating ATP while releasing carbon dioxide and water. Light respiration was found to be ca. 12 times higher than respiration in the dark, as measured using oxygen micro-sensors inside the tissue of *G. fascicularis* (Al-Horani et al. 2003a).

Although it is not tested whether this ratio changes with increasing irradiance, it is very plausible that it does. This notion would signify that the poor increase in specific growth rate compared to the increase in net photosynthetic rate is probably not due to a lack of energy, but more likely due to a lack of (nitrogen-rich) building blocks. It is therefore unlikely in this experiment that heterotrophic feeding obscured the relation between phototrophic metabolism and specific growth rate.

It is generally assumed that photosynthesis and calcification are tightly coupled and that an increase in photosynthesis will lead to an increase in calcification. Both processes follow a hyperbolic tangent function when plotted against irradiance (Barnes and Chalker 1990). However, it is not established whether both processes are saturated at the same irradiances. Comparing the studies from Houlbreque et al. (2004) and Moya et al. (2006) on *Stylophora pistillata*, provides indications that these processes are not linearly correlated. Houlbreque et al. (2004) fitted a hyperbolic tangent function to the photosynthetic rate of *S. pistillata* (nmol O_2 h^{-1} cm^{-2}) grown at 175 µE m^{-2} s^{-1} and found an I_k (i.e. subsaturation irradiance) of ~203 µE m^{-2} s^{-1} for starved corals and ~404 µE m^{-2} s^{-1} for fed corals. Moya et al. (2006) used the same coral species grown at the same irradiance and measured the calcification rate (nmol Ca^{2+} mg $protein^{-1}$ h^{-1}) at different irradiances. They found an optimal calcification rate at 100 µE m^{-2} s^{-1}. Although these studies do not use the same parameter to express their results (protein vs. surface area), these results do imply that calcification rate reaches a maximum far before photosynthetic rate does.

Our results do fit in this view, considering the fact that no direct 1:1 relation between calcification and photosynthesis was observed. Specific growth rate and net photosynthetic rate continued to deviate with increasing irradiance. If photosynthesis were to support calcification until saturation of photosynthesis, then it would be expected that a higher photosynthetic rate would lead to a higher calcification rate. To test whether maximum calcification was already reached at an intermediate irradiance level, we applied a right rectangular hyperbola function, according to the procedure of Chalker (1981) (Figure 5).

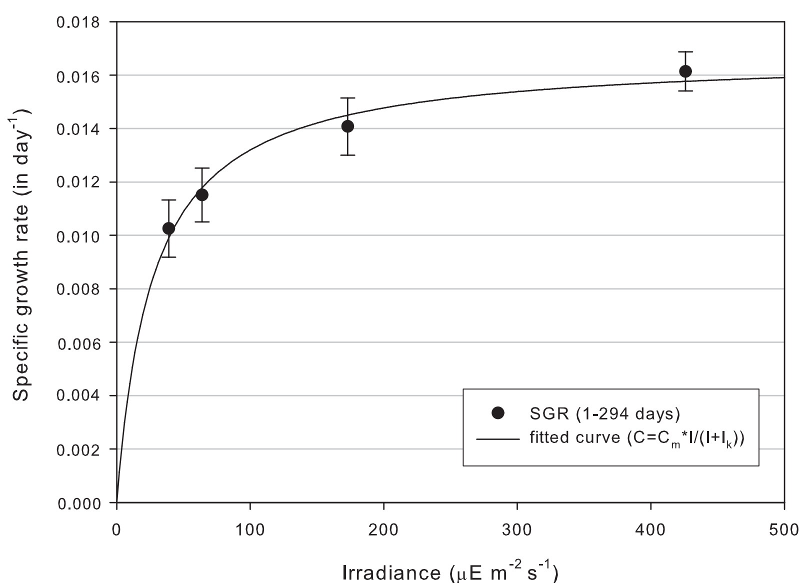

Figure 5: *Light saturation curve (right rectangular hyperbola, Chalker (1981)) fitted using Sigmaplot 8.0 on specific growth rate (day^{-1}) from day 1 to day 294 against irradiance. Values of specific growth rate are mean ± stdev, N=9.*

The fitted curve shows that skeletal growth (and hence: calcification) was close to saturation at the highest irradiance level we applied. Thus, it is not likely that calcification rate already reaches a maximum far before photosynthetic rate does, in contrast to what our comparison of the studies of Houlbreque et al. (2004) and Moya et al. (2006) suggested. Further research describing the relation between photosynthesis, calcification and irradiance in short-term experiments is in progress.

Our results confirm earlier suggestions in literature on the use of photosynthetically derived resources by corals: Moya et al. (2006) suggested that at an irradiance of 100 μE m^{-2} s^{-1} most requirements for optimizing skeletal growth (both short- and long-term) are already met and a further increase of photosynthetic rate does not add much to calcification rate. Davies (1984), Falkowski (1984) and Muller-Parker (1985) suggested that at higher irradiances, corals are not able to deal economically with their resources, and potential energy for organic matrix synthesis and calcium carbonate deposition is lost. These views suggest that at higher irradiance levels, calcification is not limited by light (and hence: photosynthesis).

It either becomes inhibited by light (e.g. Ralph et al. 1999; Winters et al. 2003) or is limited by another factor, such as the availability of bicarbonate (Marubini and Thake 1999) or the availability of planktonic food, which may be needed for the synthesis of the organic matrix (Allemand et al. 1998; Houlbreque et al. 2004). The results of the current study did not provide evidence to support the hypothesis that skeletal growth and photosynthesis are linearly correlated and therefore this hypothesis has to be rejected. Most probably linearity cannot be reached because at high irradiance, growth will be limited by other factors than irradiance.

5 Conclusion

This study demonstrates that the relationship between net photosynthesis and skeletal growth is not proportional. Thus it seems that enhancement of calcification is not entirely photosynthesis-driven: light enhanced calcification seems only to be mediated by photosynthesis at lower irradiances, while at higher irradiances the relation between calcification and photosynthesis is distorted. This finding has implications for the aquaculture of corals for aquarium/restoration purposes, since it is generally believed that more light leads to more (skeletal) growth.

The discrepancy between potential energy produced in photosynthesis and energy used for skeletal growth can be caused by several possible factors which have been discussed in this paper. Future studies should focus on the question to what extent these factors influence the relationship between photosynthesis and calcification.

Acknowledgements

This research is part of the European CORALZOO project that aims at improving coral husbandry techniques for sustainable coral breeding in zoo’s and public aquaria. In this project, scientists and aquarists collaborate to provide a scientific basis for coral husbandry techniques. The experiment described in this paper was aimed to determine the effect of irradiance on growth of scleractinian corals.

We thank the staff of Burgers Ocean for their help in setting up the experiment. This work was funded by the European Commission (Project CORALZOO-012547).

Chapter 4

Photoacclimation of the scleractinian coral *Galaxea fascicularis* to photoperiod extension under light-saturating conditions

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Abstract

Light is one of the most important abiotic factors influencing the (skeletal) growth of scleractinian corals. In order to make best use of the light available, corals are able to photoacclimate to optimize their metabolic activity under a wide range of light intensities. Whereas photo acclimation to different photon flux densities has been studied extensively, information on phenotypic adaptations of the coral host and its symbionts to changes in photoperiod remains scarce, in particular under conditions where light is not the limiting factor.

In this study, we used an experiment on coral growth where light was found not to be limiting for growth to study photoacclimation to different photoperiods. Series of nine genetically identical coral nubbins of *Galaxea fascicularis* were cultured for a period of 18 weeks at different photoperiods (8h 150 μE m^{-2} s^{-1}:16h dark, 12h 150 μE m^{-2} s^{-1}:12h dark, 16h 150 μE m^{-2} s^{-1}:8h dark, 24h 150 μE m^{-2} s^{-1}:0h dark) and different photon flux densities (8h 150 μE m^{-2} s^{-1}:16h dark, 8h 225 μE m^{-2} s^{-1}:16h dark and 8h 300 μE m^{-2} s^{-1}:16 h dark). Growth during the experiment was determined by measuring buoyant mass. To detect possible acclimation of the corals to an increased light duration (8 hours versus 16 hours), net photosynthesis, dark respiration, daily P/R ratio, zooxanthellae density and chlorophyll content were measured for colonies grown at 8h 150 μE m^{-2} s^{-1}:16h dark and colonies grown at 16h 150 μE m^{-2} s^{-1}:8h dark.

No increase in growth was detected with increasing photoperiod or irradiance. Continuous lighting (24h 150 μE m^{-2} s^{-1}:0h dark) resulted in immediate bleaching and the corals died after 14 weeks. Both net photosynthetic rate and specific growth rate were reduced compared to other studies. Daily net photosynthesis was not significantly different between the 8 hour light and 16 hour light treatment, showing photo acclimation of the corals, which might explain the comparable growth rates. No changes in chlorophyll A or zooxanthellae density were found.

Based on the results of this study it is proposed that *G. fascicularis* exhibited a form of self-shading, which is a known behavioral response of several coral species to e.g. excess light. Possibly, as a result of this photo-protective response, growth rates were not reduced when exposed to an extended photoperiod under light saturating conditions.

Keywords: Galaxea fascicularis, *light-saturation, photoacclimation, photoperiod photosynthesis*

1 Introduction

Light is one of the most important factors influencing the growth and physiology of zooxanthellate scleractinian corals due to their symbiotic relationship with phototrophic microalgae, the zooxanthellae. In the light, zooxanthellae perform photosynthesis, hereby producing oxygen and organic compounds. When their own respiratory needs are satisfied, zooxanthellae translocate the excess photosynthetic products to the coral host (Muscatine and Cernichiari 1969; Muscatine et al. 1981). Zooxanthellae can thus provide a considerable part of the resources needed for coral growth, both for soft tissue growth and for skeletal growth. The latter process is commonly referred to as light enhanced calcification. On average, calcification in light is found to be 3-4 times higher than in darkness (Gattuso et al. 1999). Although the exact mechanisms of this enhancement are still a matter of debate (Gattuso et al.1999; Furla et al. 2000; Allemand et al. 2004, Moya et al. 2006, 2008a), the importance of light for coral growth is beyond doubt.

In order to make best use of the available light, corals exhibit photoacclimation to adapt to a range of light intensities (3-15 m for *G. fascicularis*, Titlyanov and Latypov 1991; Crabbe and Smith 2006) with minimum losses in their metabolic activity (Titlyanov and Titlyanova 2002b). They may photoacclimate to maximize their photosynthetic rates under light-limiting conditions (e.g. by increasing pigmentation and/or algal density), or to protect themselves against photo inhibition and photo-oxidative stress under saturating light conditions (Levy et al. 2006). As a result of the symbiotic relationship, photo-adaptive changes can be either host-controlled and/or symbiont-controlled.

The amount of light or photons that is available to the zooxanthellae is not only determined by photon flux density, but also by the length of the photoperiod. Whereas photoacclimation to different photon flux densities has been studied extensively (Chalker et al. 1983; Iglesias-Prieto and Trench 1994; Titlyanov and Titlyanova 2002b; Anthony and Hoegh-Guldberg 2003a), information on photoadaptive changes to photoperiod remain scarce, in particular under conditions where light is not the limiting factor.

In this paper, we give a description of the effect of photoperiod extension on several photoadaptive parameters under light saturating conditions, using an experiment on coral growth and light flux where light was found not to be limiting for growth since growth did not increase with

irradiance. Series of nine genetically identical coral nubbins of *G. fascicularis* were cultured for a period of 18 weeks at different photoperiods (8h 150 μE m^{-2} s^{-1}:16h dark, 12h 150 μE m^{-2} s^{-1}:12h dark, 16h 150 μE m^{-2} s^{-1}:8h dark, 24h 150 μE m^{-2} s^{-1}:0h dark) and different photon flux densities (8h 150 μE m^{-2} s^{-1}:16h dark, 8h 225 μE m^{-2} s^{-1}:16h dark and 8h 300 μE m^{-2} s^{-1}:16 h dark). Photoacclimation of corals to different photoperiods (8 vs 16 hour light) under such light saturating conditions, was studied by measuring net photosynthetic rate, dark respiration, daily P/R ratio, zooxanthellae density and chlorophyll content. This is the first description of photoacclimation of a zooxanthellate scleractinian coral to variation in day length in a closed aquarium system.

2 Material and Methods

2.1 Experimental setup

2.1.1 Preparatory phase

Coral nubbins (single polyp clones) of *G. fascicularis* were created of colonies that were grown at a light intensity of 60 μE m^{-2} s^{-1} (70W HQI) in a closed-circuit coral aquaculture system "Quarantine system QU3" of Burgers Ocean, Arnhem, The Netherlands. QU3 is a 6000 l system consisting of four 1000 l aquaria and two 800 l sumps. The circulation system cycles 24 m^3 h^{-1} and the system is connected to a 23.5 l self made calcium reactor (pH 6.2-6.4; Q=24 l h^{-1}), and a Schuran Aquafloater AQ250 protein skimmer.

Each coral nubbin was fixed to a 7 x 7 x 0.4 cm PVC plate using Reef Construct (Aquamedic). Nine PVC plates with coral nubbins were fixed to one single square plate and maintained for four months in coral culture system QU3 at an irradiance of 150 μE m^{-2} s^{-1} which was provided by ATI lighting armatures containing 10.000K T5 Coral Light (Korallenzucht) bulbs. A light:dark cycle of 10L:14D was applied.

2.1.2 Experimental phase

After 7 months (28 weeks), each plate containing 9 coral nubbins was assigned to each of the following light regimes (see also Table 1): 8h 150 μE m^{-2} s^{-1}:16h dark, 12h 150 μE m^{-2} s^{-1}:12h dark, 16h 150 μE m^{-2} s^{-1}:8h dark, 24h 150 μE m^{-2} s^{-1}:0h dark, 8h 225 μE m^{-2} s^{-1}:16h dark and 8h 300 μE m^{-2} s^{-1}:16 h dark. In addition, 3 coral nubbins were put in the same conditions for experimental work within respirometric flow cells. To facilitate adaptation to the new light regimes, all regimes were adapted in gradual steps (max 100 μE m^{-2} s^{-1} per day or 2 hours difference per day) during a time span of 7 days.

Lighting was provided by six ATI lighting armatures containing 10.000 K T5 Coral Light (Korallenzucht) bulbs and adjusted to irradiance and light duration using a Profilux aquarium computer. Irradiance was measured weekly at the same distance from the light source as the corals and adjusted if needed. Average values per treatment are shown in Table 1.

Table 1: *Description of experimental treatment (irradiance, photoperiod and daily light flux) and the average values for irradiance and water flow that were measured during the 18 week experimental period in each experimental treatment. Note: differences in water flow between treatments were not significantly different (p>0.10).*

	Irradiance (in μE m^{-2} s^{-1})	**Photoperiod** (hours light:dark)	**Average irradiance** (in μE m^{-2} s^{-1})	**Average water flow** (in cm s^{-1})
Treatment 1	150	8L:16D	149.7 ± 2.7	15.3 ± 3.5
Treatment 2	150	12L:12D	149.8 ± 4.4	16.3 ± 3.1
Treatment 3	150	16L:8D	151.0 ± 5.9	16.2 ± 2.6
Treatment 4	150	24L:0D	151.5 ± 6.2	15.2 ± 3.2
Treatment 5	225	8L:16D	223.0 ± 3.9	14.9 ± 2.6
Treatment 6	300	8L:16D	298.1 ± 6.2	16.9 ± 2.8

Experiments were done in semi-enclosed compartments of Q3 system to prevent lighting from one treatment to contaminate the other. As a consequence, no free movement of the water surface between experimental treatments and the overflow was possible. Skim

boxes, connected to powerful circulation pumps (Aqua Medic Ocean runner 3500 and 6500, resp. 3500 and 6500 l h^{-1}), were installed and adjusted individually to keep the water surface free from algae. The water volume in each experimental treatment (circa 75 l) was estimated to be replaced every 5 minutes.

Within each experimental treatment, water flow was created by two small Eheim pumps (Type 1002; 1000 l h^{-1}) connected to a perforated PVC pipe. Flow velocity was measured weekly using a SENSA-RC2 electromagnetic current meter (Aquadata) at the location of the corals inside each experiment treatment (Figure 1) and the average value was maintained around 15 cm s^{-1}. Average values per treatment are shown in Table 1.

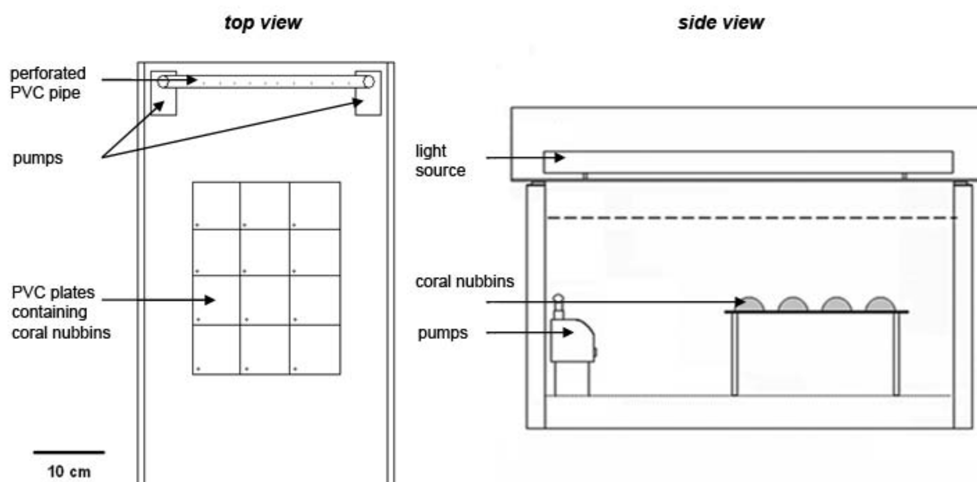

Figure 1: *Top and side view of an experimental treatment, showing the two Eheim pump sconnected by a perforated PVC pipe for creating water flow and the location of the nine coral nubbins inside the treatment. The dashed line indicates the water level inside the experimetnal treatment. For clarity, skim boxes are omitted from these figures.*

Each experimental treatment was fed indirectly by daily feeding of the entire coral culture system (4000-8000 artemia l^{-1}) and additionally twice a week directly inside each experimental treatment (approx. 250 artemia l^{-1}). Artemia were hatched on site and subsequently enriched using Easy DHA Selco for 24 hours. Seawater was made up from Tropic Marine salt (Zoomix without bromide).

Temperature in the system was maintained at 25.8 ± 0.3 S.D. °C, salinity at 34.1 ± 0.1 S.D. ppt and pH 8.1 ± 0.1 S.D. Water quality parameters were monitored on regular basis. During the experiment, the alkalinity in the system was 4.6 ±1.0 S.D. mEq l^{-1}, calcium concentration 395.9 ± 17.7 S.D. mg l^{-1}, magnesium concentration 1203 ± 63.7 S.D. mg l^{-1}, nitrate concentration 0.195 ± 0.077 S.D. mg NO_3^--N l^{-1}, phosphate concentration 0.018 ± 0.015 S.D mg PO_4^{3-} l^{-1}.

2.2 Growth parameters

Growth was measured as an increase in buoyant mass according to Schutter et al. (2008). It should be noted that buoyant mass is not a good approximation of skeletal mass, since the net upward buoyancy force (i.e. the weight of seawater displaced by the coral wehn weighing it under water) will result in measuring only a fraction of the absolute mass (i.e. a fractional mass). To convert buoyant mass into skeletal mass, one needs to know the density of the seawater and the volume of the coral. Moroever, one needs to assume a constant density of the coral skeleton during the experiment. However, for the purpose of this study, knowledge of the magnitude of buoyant mass was sufficient to calculate growth rates.

Specific growth rates for buoyant mass were calculated between week 4 and week 18 (in weeks after the adjustment to the new light regimes), since it was assumed that corals need approximately 4 weeks to adapt to a new light regime (Falkowski and Dubinsky 1981; Anthony and Hoegh-Guldberg 2003ab). Assuming exponential growth, specific growth rates (μ) were calculated using the formula:

$$\mu = (\ln BW_n - \ln BW_{n-1})/\Delta t \qquad [day^{-1}]$$

where μ is the specific growth rate (day^{-1}), BW_n is buoyant mass at the end of a growth interval, BW_{n-1} is buoyant mass at the start of a growth interval and Δt is time between measurements of buoyant mass in this growth interval.

2.3 Respirometric measurements

2.3.1 Net photosynthesis and respiration

Net photosynthesis and dark respiration were determined for corals maintained at a photoperiod of 8 hours light and at a photoperiod of 16 hours light (irradiance: 150 μE m^{-2} s^{-1}). Three coral colonies of each treatment were measured on three different days by means of intermittent flow respirometry in a 1616 ± 5 ml respirometric flow cell, according to Schutter et al. (2008).

Net photosynthetic oxygen production was measured at an irradiance of 150 μE m^{-2} s^{-1} (i.e. corresponding to the irradiance in the experimental treatment). Respiratory consumption of oxygen was measured in the dark. Lighting was provided by a T5 lighting system (ATI) containing eight 24 W coral light bulbs (Korallenzucht). A flow speed of ±10 cm s^{-1} was applied to ensure adequate mixing for respirometry.

2.3.2 Daily P/R ratios

Daily P/R ratios were calculated to indicate whether the corals were self-supporting with respect to carbon. The ratios indicate whether the coral could satisfy their daily respiratory needs using photosynthetic products translocated by their zooxanthellae only (Muscatine et al. 1981). Values greater or equal than 1 indicate that corals are self-sufficient with respect to carbon, while values lower than 1 indicate that they are not and that they need other sources of carbon to sustain their respiratory needs. To be able to calculate daily P/R ratios, net photosynthetic and dark respiratory rates were converted to carbon equivalents, using the following equations:

$$P_c = P_{net} \times (12/32) \qquad [\mu mol\ C\ min^{-1}\ cm^{-2}]$$

$$R_c = R_{dark} \times (12/32) \qquad [\mu mol\ C\ min^{-1}\ cm^{-2}]$$

where P_c is net photosynthetic rate in carbon equivalents (μmol C min^{-1} cm^{-2}), P_{net} is net photosynthetic rate in oxygen equivalents (μmol O_2 min^{-1} cm^{-2}) and the factor (12/32) is

the molar conversion factor to convert oxygen equivalents (O_2) to carbon equivalents (C), Analogously, R_c is dark respiratory rate carbon equivalents (µmol C min^{-1} cm^{-2}) and, R_{dark} is the dark respiratory rate in oxygen equivalents. Since we do not know the exact composition of substances that are produced during photosynthesis and that are respired during respiration, no further corrections were applied using metabolic quotients (Gattuso and Jaubert 1990).
Daily P/R ratios were calculated using the following equation:

$$\text{Daily P/R ratio} = (P_c * L) / (R_c * D) \qquad \text{[dimensionless]}$$

where P_c and R_c are expressed in mg C $hour^{-1}$ cm^{-2}, and L and D correspond respectively to the number of hours of light and dark per day. Although interpretation of daily P/R ratios that are derived from short term measurements is not justified according to Muscatine et al. (1981), it is used here as an approximation.

2.4 Analysis of coral tissue

2.4.1 Tissue removal

At the end of the experiment, corals were removed from their treatments, snap-fixed in formaldehyde (3 minutes 10% formaldehyde in 0.22 µm filtered seawater (FSW) 34ppt), rinsed shortly in 0.22 µm FSW 34 ppt, wrapped in tin foil and frozen at -20°C until further processing (Broadbent et al. 2002).
Corals (n=9) from two treatments (8 hour light/150 µE m^{-2} s^{-1} and 16 hour light/150 µE m^{-2} s^{-1}) were taken from the freezer and soaked in Ca^{2+}-Mg^{2+}-free artificial seawater (ASW) with ethylene diamine tetracetic acid (EDTA) in a slowly moving water bath at 50°C overnight in order to facilitate tissue removal. This solution was prepared according to Rinkevich et al. (2005). Tissue was removed the next day using high pressured N_2 (max 1.5 bar within plastic bag). Cell suspensions were collected, diluted with 34 ppt artificial sea water (ASW) and centrifuged three times for 10 minutes at 4°C at 4000 rpm. The final tissue pellets of each coral were collected in one tube and total volume was determined

using a 5 ml pipette. After homogenization using a LABOCAT X1030, samples were taken to count the number of zooxanthellae (200 µl) and for chlorophyll analysis (1 ml).

2.4.2 Chlorophyll analysis and zooxanthellae count

Chlorophyll was extracted by adding 9 ml 100% acetone to 1 ml tissue homogenate and storing it at -20°C overnight. The next day, this suspension was homogenized again using a LABOCAT X1030. After settlement of the pellet, the absorbance of the extract was measured in triplicate using a Beckman Coulter DU 530 Spectrophotometer at 750, 664 and 630 nm. 90% acetone in demiwater was used as a blank. The concentrations of chlorophyll A and chlorophyll C_2 were computed according to the equations given by Jeffrey and Humphrey (1975) for dinoflagellates. Each extinction value (OD664 and OD630) was corrected for the absorbance at 750 nm, which is a correction for the turbidity of the sample.

Zooxanthellae were counted using a Bürker-Turk counting chamber. Zooxanthellae density was expressed in amount of zooxanthellae per cm^2 surface area. Using the chlorophyll data, the amount of chlorophyll per zooxanthellae was also calculated.

2.5 Data analysis

Normality ($p>0.05$) and homogeneity of variance ($p>0.05$) of the growth and respirometry data were tested using Shapiro-Wilk and Levene's test in SAS 9.1. Since our growth, photosynthesis and respiration data did not satisfy the assumptions for ANOVA testing, we used Kruskall Wallis as a non-parametric test to detect statistical differences between treatments.

A Student T-test was used to detect statistical differences in chlorophyll content and zooxanthellae density between the 8 hour light and 16 hour light treatment.

3 Results

3.1 Effect of irradiance and photoperiod on growth

3.1.1 Photoperiod

Specific growth rate did not change with increasing light duration (8 hours, 12 hours, 16 hours). The corals in the 24 hours light treatment started bleaching after the change to the new light regime, but managed to stay alive and to keep growing until week 8. They died finally after week 14 due to overgrowth with algae. Their specific growth rate (4-14 weeks) was significantly lower than the corals in the other light treatments ($p \leq 0.0005$) (Figure 2).

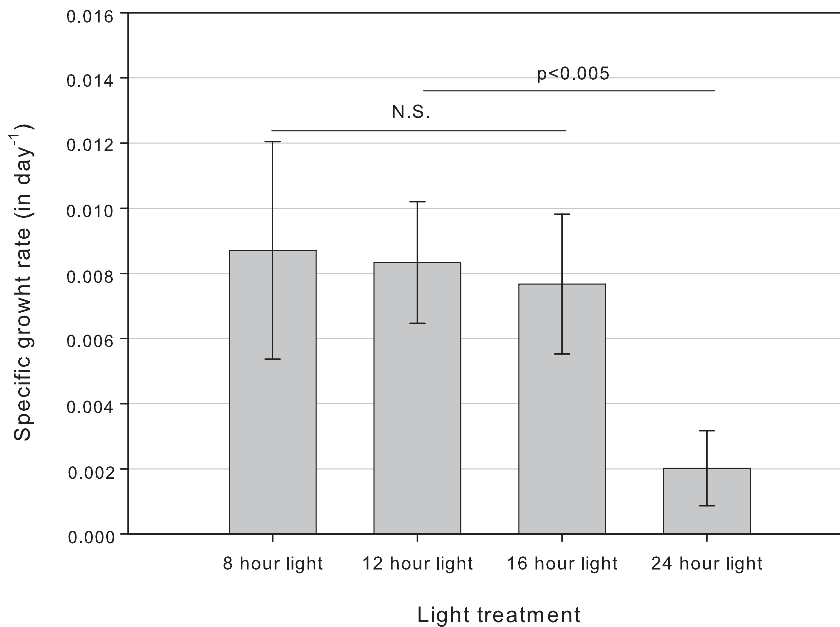

Figure 2: *Effect of photoperiod on the specific growth rate in terms of buoyant mass of* G. fascicularis *between week 4 tot week 18 of the experiment. Irradiance was kept at 150 µE m^{-2} s^{-1}, so the different light treatments received a daily light input of resp. 4.32 E m^{-2} day^{-1}, 6.48 E m^{-2} day^{-1}, 8.64 E m^{-2} day^{-1} and 12.96 E m^{-2} day^{-1}. Values are mean ± S.D. n=9.*

3.1.2 Irradiance

Specific growth rate was not found to increase with increasing irradiance (150 µE m^{-2} s^{-1}, 225 µE m^{-2} s^{-1}, 300 µE m^{-2} s^{-1}). The corals in the 225 µE m^{-2} s^{-1} and 300 µE m^{-2} s^{-1} light treatment had a significant lower specific growth rate as buoyant mass compared to the 150 µE m^{-2} s^{-1} light treatment ($p<0.002$)(Figure 3).

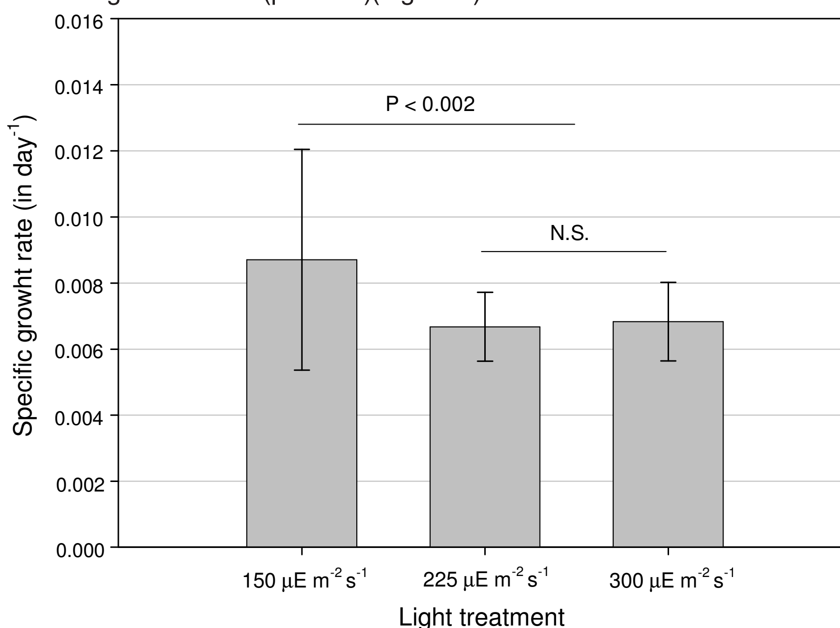

Figure 3: *Effect of irradiance on the specific growth rate in terms of buoyant mass of* G. fascicularis *between week 4 tot week 18 of the experiment. Photoperiod was kept at 8L:16D, so the different light treatments received a daily light input of resp. 4.32 E m^{-2} day^{-1}, 6.48 E m^{-2} day^{-1} and 8.64 E m^{-2} day^{-1}. Values are mean ± S.D. n=9.*

3.2 Respirometric measurements

Average net photosynthetic rate (in µmol O_2 min^{-1} cm^{-2}) was significantly higher for corals in the 8 hour light treatment compared to the 16 hour light treatment ($p=0.004$, Table 2), while average dark respiratory rate was not significantly different ($p=0.40$).

Despite the fact that the corals in the 16 hours light treatment are twice as long exposed to light compared to the corals in the 8 hour light treatment, the total amount of oxygen produced per day (i.e. daily net photosynthesis) was found to be not significantly

different between treatments ($p=0.40$). However, the total amount of oxygen respired in the night was significantly higher for the corals in the 8 hour light treatment ($p=0.003$). Consequently, the average daily P/R ratio was significantly higher for the corals in the 16 hour light treatment ($p=0.009$), i.e. the average daily P/R ratio for the corals in the 16 hour light treatment was above 1 (1.59 ± 0.66 S.D), while the average daily P/R ratio for the corals in the 8 hour light treatment was below 1 (0.90 ± 0.44 S.D).

Table 2: *Overview of respirometric parameters of corals maintained at a photoperiod of 8 hour light (8h 150 μE m^{-2} s^{-1}: 16 dark) and at a photoperiod of 16 hour light (16h 150 μE m^{-2} s^{-1}: 8h dark). Values are mean ± S.D. P-values are given (one way ANOVA or Kruskall-Wallis test). N=9 observations for 8 hour light treatment, N= 5 observations for 16 hour light treatment.*

		8 hour light		16 hour light		
		mean	S.D.	mean	S.D.	p-value
net photosynthesis	μmol O_2 $min^{-1}cm^{-2}$	0.024	0.007	0.013	0.005	0.004
dark respiration	μmol O_2 $min^{-1}cm^{-2}$	-0.017	0.006	-0.020	0.005	0.27
daily net photosynthesis	μmol O_2 cm^{-2} day^{-1}	11.627	3.419	12.756	4.799	0.40
daily respiration	μmol O_2 cm^{-2} day^{-1}	-16.303	5.414	-9.455	2.198	0.003
daily P/R ratio		0.902	0.438	1.585	0.659	0.0093

3.3 Chlorophyll and zooxanthellae

No significant difference in chlorophyll A content (in μg Chl A cm^{-2}) and chlorophyll C_2 content (in μg Chl C_2 cm^{-2}) was detected between the corals in the 8 hour light and 16 hour light treatment (T-test, resp. $p=0.47$ and $p= 0.45$), despite visual observation suggesting that the corals in the 16 hour light treatment were less pigmented.

Zooxanthellae density (zoox cm^{-2}) and amount of Chlorophyll per zooxanthellae (Chl A $zoox^{-1}$) were also not significantly different between the corals in the 16 hour light treatment and the corals in the 8 hour light treatment (Table 3).

Table 3: *Overview of chlorophyll and zooxanthellae measurements of corals maintained at a photoperiod of 8 hour light (8h 150 µE m^{-2} s^{-1}: 16 dark) and at a photoperiod of 16 hour light (16h 150 µE m^{-2} s^{-1}: 8h dark). Values are mean ± S.D. P-values are given (student's T-test). N=9 for chlorophyll, N=5 for zooxanthellae.*

		8 hour light		16 hour light		
		mean	S.D.	mean	S.D.	p-value
Chlorophyll A	µg cm^{-2}	3.66	1.81	4.21	1.31	0.47
Chlorophyll C_2	µg cm^{-2}	1.12	0.52	1.31	0.51	0.45
Zooxanthellae density	zoox cm^{-2}	$2.58x10^6$	$9.85x10^5$	$3.17x10^6$	$7.69x10^5$	0.32
Chlorophyll A per zooxanthella	µg $zoox^{-1}$	1.81	0.67	1.54	0.67	0.57

4 Discussion

4.1 Coral growth: a matter of photons?

No increase in specific growth rate was found with increasing irradiance or photoperiod. Continuous lighting (24h 150 µE m^{-2} s^{-1}:0h dark) resulted in immediate bleaching and death after 14 weeks. The specific growth rate of these corals was significantly decreased compared to the other treatments. Since previous studies demonstrated that growth of scleractinian corals increases with increasing irradiance (Marubini et al. 2001; Reynaud-Vaganay et al. 2001; Reynaud et al. 2004; Schutter et al. 2008), it can be concluded that light was not limiting in this study. Rather it seems that light was in excess, since the corals in the highest light treatment (300 µE m^{-2} s^{-1}) exhibited a significantly lower specific growth rate than the corals lowest light treatment (150 µE m^{-2} s^{-1}). Obviously, the availability of photons alone cannot enhance coral growth. Besides the amount of photons, also other factors may play a role in determining coral growth rate. Factors known to be limiting for the growth of stony corals are e.g. water flow (Lesser et al. 1994), aragonite saturation state (Gattuso et al. 1998; Leclercq et al. 2000; Schneider and Erez 2006) and its associated components (Marubini et al. 2008), the availability of essential trace metals such as copper and zinc (Ferrier-Pagès et al. 2005) and/or the availability of essential nutrients such as

aspartic acid (Allemand et al. 1998a) that are mostly supplied by heterotrophic feeding (Houlbreque and Ferrier-Pagès 2009). While factors known to have a inhibiting effect on coral growth are e.g. elevated nutrient concentrations (Ferrier-Pagès et al. 2000; Fabricius 2005), increased iron concentration (Ferrier-Pagès et al. 2001), increased temperatures (Jokiel and Coles 1990, Marshall and Clode 2004), competition (Rinkevich and Loya 1985, Tanner 1995) and sedimentation (Rogers 1990). The fact that the specific growth rate of *G. fascicularis* grown at 8h 150 μE m^{-2} s^{-1}:16h dark was noticeably lower compared to *G. fascicularis* grown at 10h 166 μE m^{-2} s^{-1}:14h dark during a similar time period in a previous study (0.0087 ± 0.0033 day^{-1} versus 0.0130 ± 0.0011 day^{-1}, based on data Schutter et al. (2008) suggests that one or more factors were limiting or inhibiting. However, the factor(s) limiting or inhibiting coral growth in this study could not be conclusively determined.

4.2 Photo-acclimation to prolonged light duration under light-saturating conditions

Since light was not limiting, corals in the 16 hour light treatment received excess light. Despite receiving excess light, the corals in the 16 hour light treatment managed to retain growth rates comparable to the corals in the 8 hour light treatment, i.e. there was no photo-inhibition of growth. Therefore, some form of photo adaption must have played a role. Our respirometric data demonstrate that the corals in the 16 hour light treatment adapted to a longer photoperiod by decreasing the hourly rate of photosynthesis compared to the corals in the 8 hour light treatment. As a result, daily net photosynthesis was not significantly different between treatments, which is in agreement with their similar specific growth rates. This result fits in the view that enhancement of calcification is mediated by photosynthesis (Gattuso et al. 1999; Allemand et al. 2004), since neither growth nor daily net photosynthesis were significantly different between the two treatments. However, the daily P/R ratio was significantly different between treatments (1.59 ± 0.66 S.D for 16 hour light, 0.90 ± 0.44 S.D for 8 hour light), indicating that the corals in the 16 hour light treatment potentially had more access to photosynthetic products translocated by their zooxanthellae to satisfy their daily respiratory needs (Muscatine et al.1981) and to

generate ATP that can be used for calcification. Despite having both a higher availability of photosynthetic carbon (i.e. higher daily P/R ratio) and a longer time period during which the intracellular saturation state of calcium carbonate is positively modified by photosynthesis, this did not make a difference for the growth of the corals in the 16 hour light treatment. This could be explained by an increasing need for defense mechanisms (e.g. free radical scavenger enzymes, Levy et al. 2006) against harmful, reactive oxygen species that are produced as a result of excess light in the 16 hour light treatment. The increased availability of photosynthetic carbon might therefore have been allocated towards defense mechanisms against photo-oxidative stress instead of skeletal growth, explaining the absence of increased growth with increasing availability of photosynthetic carbon. Energy allocation to photo-protective mechanisms remains to be studied in future investigations.

Based on zooxanthellae density and chlorophyll content, it is not possible to distinguish whether the adaption (photo-acclimation) to the longer light duration was host- or symbiont-controlled, since neither differences in zooxanthellae density nor in chlorophyll content were found. Generally, corals acclimate to increased light by regulating their light capture. This can occur either by limiting light harvest and utilization of their photosystems (i.e. by decreasing the amount of photosynthetic pigments per zooxanthellae, decreasing the zooxanthellae density in polyp tissue, or increasing non-photochemical quenching) and/or limiting light capture by self-shading of their photosynthetic surfaces (ie. by changes in morphology and anatomy of coral colony) (Titlyanov et al. 2000; Anthony et al. 2005). Self-shading can be either a morphological response (i.e. expressed in colony architecture, long-term response) or a behavioral response (i.e. expressed as tissue retraction, short-term response). Tissue retraction is often a response to stress, e.g. in response to sub-aerial exposure, bright light or increased iron concentrations (Brown et al. 1991) and can be expressed as polyp retraction or withdrawal of tentacles (Brown et al. 1994; Brown et al. 2002). It is also known to occur in *G. fascicularis* (Brown et al. 1994). Since self-shading does not involve the loss of either zooxanthellae or photosynthetic pigments (Brown et al. 1994), it is possible that this occurred in the present study. Moreover, due to the sudden change in light regime, the corals in the present study neither had the time

for morphological changes of the skeleton that normally occur during growth in a certain light regime (Anthony et al. 2005). The reduced photosynthetic rates might therefore be explained by lower light levels as a result of self-shading, and are hence host-controlled. Tissue retraction is likely an effective mechanism to keep irradiance within a physiologically optimal range, just like self-shading through morphological plasticity of skeletal architecture (Anthony et al. 2005),

The mechanism to adapt to excess light might be the same for both excess light received as irradiance and as light duration. In response to excess irradiance, in general corals will engage in mechanisms for photo-protection and limit their light capture to prevent photo inhibition, which in either way (limited light or photo-inhibition) will result in reduced photosynthetic rates (Titlyanov et al. 2000; Anthony et al. 2005). The same was found for excess irradiance as light duration. The only difference might be in the time of onset of photo-adaptation or photo-inhibition, since if the irradiance itself is not stressful, the amount of photons can accumulate to stressful amounts during the day. However, this remains to be demonstrated.

The growth and physiological response of corals to increased light duration under light limiting conditions remains to be investigated. The use of photosynthesis-irradiance curves and/or PAM (pulse-amplitude modulation) measurements during day will provide more insight into the photo-acclimative responses.

5 Conclusion

Coral growth is not only a matter of available photons. Under the given experimental conditions, no positive correlation between light availability and growth was observed, neither with increasing photoperiod, nor with increasing irradiance. This indicates that light was not the limiting factor and was most probably in excess. Continuous lighting (24 h) resulted in immediate bleaching and finally death of the corals.

Corals were able to adapt to prolonged light duration under light saturating conditions by decreasing their hourly rate of photosynthesis. As a result, daily net photosynthesis was not significantly different between corals grown at 8 hours light and 16 hours light. This

result fits in the view that enhancement of calcification is mediated by photosynthesis, since neither growth nor daily net photosynthesis were significantly different between the two treatments. Photoacclimation to prolonged photoperiod was not achieved by changes in zooxanthellae density or chlorophyll content. It is proposed that the corals exhibited a form of self-shading that reduced the amount of photons reaching the coral, thereby reducing their photosynthetic rates and specific growth rates.
The growth and physiological response of corals to increased light duration under light limiting conditions still remains to be investigated.

Acknowledgements

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Chapter 5

Modification of light utilization for skeletal growth by water flow in the scleractinian coral *Galaxea fascicularis*

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Abstract

In this study we tested the hypotheses whether water flow stimulates (skeletal) growth more at high irradiance than at intermediate irradiance, and whether such effect is mediated by a water flow modulated effect on photosynthesis. Four series of nine nubbins of *Galaxea fascicularis* were grown at either high (600 μE m^{-2} s^{-1}) or intermediate (300 μE m^{-2} s^{-1}) irradiance in combination with either high (15-25 cm s^{-1}) or low flow (5-10 cm s^{-1}). Growth was measured as buoyant mass and surface area. Photosynthetic rates were measured at each coral's specific experimental irradiance and flow speed. Water flow stimulated (skeletal) growth more at high irradiance than at intermediate irradiance. Enhancement of coral growth with either increasing water flow or increasing irradiance could not be explained by net photosynthetic rates. Possibly, the need for costly photo-protective mechanisms at low flow regimes can explain the differences in growth with flow.

Keywords: *light, water flow, light-flow interaction,* Galaxea fascicularis*, skeletal growth, photosynthesis*

1. Introduction

Light and water flow are two of the most important factors influencing the growth of scleractinian corals. Light stimulates coral growth by the process of light-enhanced calcification (Gattuso et al. 1999; Allemand et al. 1998) which is mediated by zooxanthellar photosynthesis. Consistent with this hypothesis, a higher photon flux density increases net photosynthetic rate and long-term skeletal growth (Marubini et al. 2001; Reynaud-Vaganay et al. 2001; Reynaud et al. 2004; Schutter et al. 2008). These increases might be different at different flow velocities. Water flow reduces the diffusive boundary layer around the coral. This layer acts as barrier to the supply and uptake of dissolved gasses, nutrients and heterotrophic food but also as a barrier to the removal of sediment and metabolic waste products such as oxygen, oxygen radicals and possibly mucus. Possibly because one or more of the above mechanisms, increasing water flow rates correlate with increased skeletal growth (Yokiel 1978; Montebon and Yap 1997; Sebens et al. 2003; Nakamuraet al. 2005).

Both abiotic factors have only been studied separately, while they might interact strongly (Lesser et al. 1994). At high irradiance and with higher photosynthetic rates, there will be a higher need for the removal of photosynthetically produced oxygen (Finelli et al. 2006; Finelli et al. 2007) and the supply of inorganic carbon (Lesser et al. 1994). Thus, the thickness of the boundary layer needs to be smaller at higher irradiance than at lower irradiance and consequently water flow needs to be higher at the higher irradiance level. To investigate this interaction, the influence of water flow on light utilization for skeletal growth of the scleractinian coral *G. fascicularis* was studied using a factorial design. To test whether the observed effects on coral growth are related to the influence of water flow on net photosynthesis, photosynthetic rates were determined of three coral colonies from each experimental treatment by measuring them at their specific irradiance and flow speed inside a respirometric flow cell.

2. Material and Methods

2.1 Study species

Thirty-six (36) coral nubbins (single polyp clones) of *G. fascicularis* were created of colonies that were grown at an irradiance of 60 µE m^{-2} s^{-1} (70W HQI) in a close-circuit coral aquaculture system in Burgers Ocean, Arnhem, The Netherlands. Each coral nubbin was fixed to a 7 x 7 x 0.4 cm PVC plate using Reef Construct (Aquamedic). Nine PVC plates with coral nubbins were randomly fixed to each of four square pegboards. After recovering for one week, they were transported to a 600 liter closed-circuit coral culture system at Wageningen University.

2.2 Coral culture system

The coral culture system in Wageningen consisted of a 400 l aquarium and a 200 l sump containing life rock. The system was connected to an ATI protein skimmer and a self-assembled calcium reactor (6.2-6.4 pH, coral sand). Seawater was made up from Reef Crystals®. Temperature was maintained at 26.1 ± 0.4 °C and salinity at 34.4 ± 1.5 ppt. Water quality during experimental time was measured at regular intervals and maintained. Alkalinity in the system over the course of the experiment was 3.3 ± 1.1 S.D. mEq l^{-1}, calcium concentration 388.1 ± 38.4 S.D. mg Ca^{2+} l^{-1}, magnesium concentration 1348.8 ± 77.6 S.D. mg Mg^{2+} l^{-1}, nitrate concentration between 0 and 2 mg NO_3^- l^{-1} and phosphate concentration between 0 and 0.5 mg PO_4^{3-} l^{-1}.

2.3 Experimental setup

Each pegboard containing 9 nubbins was assigned to each of the following experimental treatments: high irradiance x high flow (HI x HF), high irradiance x low flow (HI x LF), intermediate irradiance x high flow (II x HF) and intermediate irradiance, low flow (II x LF). During the experiment, the intermediate light regime was kept at an irradiance of approx. 300 µE m^{-2} s^{-1} and the high light regime was kept at an irradiance of approx. 600 µE m^{-2} s^{-1}. Water flow was kept between 15-25 cm s^{-1} for high flow, and between 5-10 cm s^{-1} for low flow.

Lighting was supplied using two ATI lighting armatures that each contained four 39 W ATI Aquablue Spezial bulbs. A light:dark cycle of 12L:12D was applied. Irradiance was measured using a Li-Cor 192SA quantum underwater sensor at the same distance from the light source as the corals were located. A broad stream of unidirectional water flow was created by connecting two Tunze TURBELLE ® nanostream 6055 pumps with a perforated PVC pipe and controlled using a Tunze 7095 Multicontroller. A flow straightener was placed after the first set of corals in the "high flow" treatment, so to reduce the flow that reached the corals in the "low flow" treatment (Figure 1). Water flow was measured in the absence of the corals by placing the SENSA RC-2 electro-magnetic velocity meter (Aquadata) at the exact position of the corals. The corals were fed 7 days a week with approx. 150.000 freshly hatched *Artemia* nauplii each day, yielding concentrations of 250 *Artemia* per liter in the rearing system.

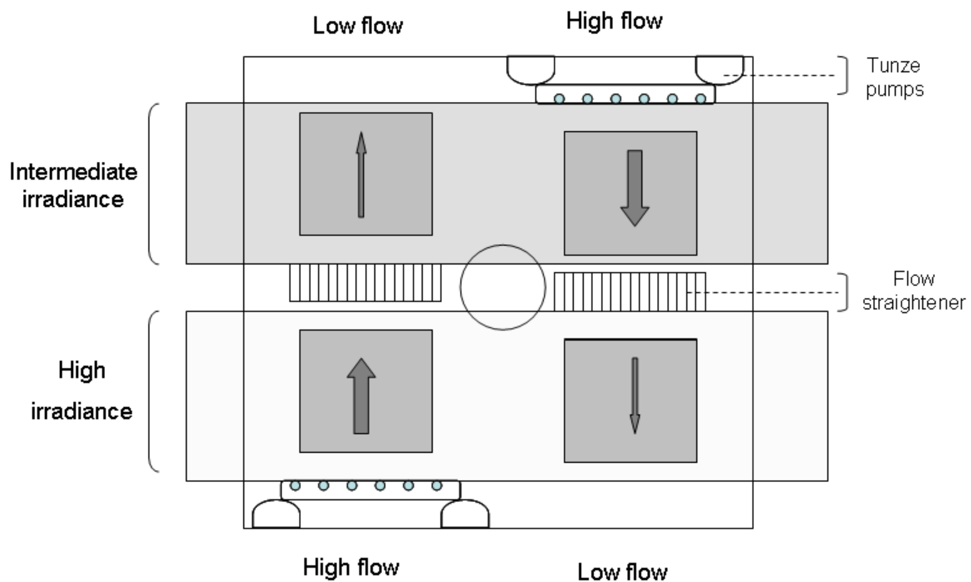

Figure 1: *Schematic drawing of the top view of the experimental setup of the light x flow experiment. Water flow was created at the high flow side (thick arrows) and reduced by a flow straightener to reach a lower flow rate at the low flow side (thin arrows). The lighting armatures were placed perpendicular to the water streams.*

2.4 Growth parameters

Growth was measured as an increase in buoyant mass and surface area according to Chapter 2. Determination of buoyant mass (known as “ the buoyant weighing technique”) is a good method to measure skeletal growth, since coral tissue has a density which is similar to that of seawater and therefore does not contribute significantly to the buoyant mass of the coral. Tissue only comprises 1% of the total buoyant mass when tissue does not penetrate deep into the skeleton (Davies 1989). Moreover, buoyant weighing is a simple and non-destructive technique, allowing long-term monitoring of skeletal growth. It should be noted that buoyant mass is not a good approximation of skeletal mass, since the net upward buoyancy force (i.e. the weight of seawater displaced by the coral wehn weighing it under water) will result in measuring only a fraction of the absolute mass (i.e. a fractional mass). To convert buoyant mass into skeletal mass, one needs to know the density of the seawater and the volume of the coral. Moroever, one needs to assume a constant density of the coral skeleton during the experiment. However, for the purpose of this study, knowledge of the magnitude of buoyant mass was sufficient to calculate growth rates.

Surface area was measured as projected surface area. Pictures were taken perpendicular to the coral directly inside the aquarium system using a Nikon Coolpix S1 5.1 mp digital camera in a Nikon WP-CP5 underwater housing. Surface area was determined by image analysis using ImageJ (1.37v) by tracing the live part of the coral colony. Since tentacle extension is variable over time, no tentacles that were extending beyond the skeleton were traced for surface area. Pictures were also taken of the coral nubbins before fixation to their PVC plates. Specific growth rates were calculated using the formula:

$$SGR = \ln (BM_n / BM_{n-1})/\Delta t \qquad [\text{day}^{-1}]$$

where SGR is the specific growth rate (day^{-1}), BM_n is buoyant mass (or surface area) at the end of the experiment, BM_{n-1} is buoyant mass (or surface area) at the start of the experiment and Δt is time between the measurements of buoyant mass.

Since our observations suggested that the corals suffered from growth retardation by an unknown cause during the first part of the experiment (1-125 days, in total 125 days), it was decided to use only the growth data from the second part of the growth experiment (125-400 days, in total 275 days) for analysis. Increase in buoyant mass and surface area were calculated from this moment, and specific growth rates were calculated between this moment and the end of the experiment. The size of the coral colony at the start of the second part of the experiment was taken as covariate (see Data Analysis).

2.5 Respirometric measurements

Net photosynthetic rate was measured of three different corals from each experimental treatment inside a respirometric flowcell (3500 ml, Figure 2), which allowed measurement of net photosynthesis at exactly the same irradiance and flow speed as the corals experienced in the experiment. The irradiance applied was either 280 µE m^{-2} s^{-1} or 560 µE m^{-2} s^{-1} (corresponding to ambient irradiance at that time) and water flow speed either 5 cm s^{-1} or 20 cm s^{-1} (low flow vs high flow).

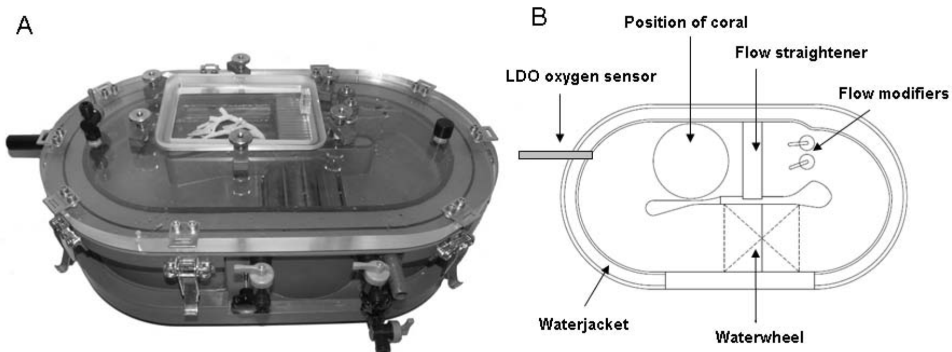

Figure 2: *Respirometric flowcell (version III).* ***a)*** *Picture of the top/side view of the respirometric flowcell.* ***b)*** *schematic drawing of top view of respirometric flowcell, indicating the location of the different parts.*

Water flow speeds were created using a modified paddlewheel that was powered by a Maxon DC motor with a 3-channel incremental encoder and line driver that allows precise control of rotational speed. Water flow speeds across the coral section were calibrated

using video recordings (30 fps, 480 x 860 pixels) of *Artemia* cysts (Ø max. 500 µm) moving in a 5 mm light plane created by a slide projector with a slitted cover across the lens. Video recordings were analyzed using a tailor-made program for particle tracing in Matlab, which allowed less time-consuming and more efficient tracing of particles across successive frames. Only particles that remained in the beam plane for 4 or more successive frames were used for calculations. Lighting was provided by a T5 lighting system (ATI) containing eight 24 W Aquablue Spezial bulbs. Irradiance was measured submerged in seawater and underneath the plastic lid, at the exact same distance from the light source for each coral.

Each incubation with a coral was preceded by a control incubation (seawater only). The increase in oxygen concentration was measured every 10 seconds using a luminescent oxygen probe (Hach) until a difference in concentration was detected of ±1 mg O_2 l^{-1}. Seawater was replaced after each measurement with a coral, to prevent potential effects of hyperoxia or hypoxia on the performance of the experimental corals and to remove possible accumulated waste products.

Temperature inside the respirometric cell was maintained at 26 °C and salinity at 35 ppt. Surface area and volume were determined of each coral in order to normalize the respirometric data. Photosynthetic and respiratory rates were calculated according to Chapter 2.

2.6 Data analysis

Normality (p>0.05) and homogeneity of variance (p> 0.05) of the data were tested using Shapiro-Wilk and Levene's test in SAS 9.1. A two-way analysis of covariance (ANCOVA) was used to test the main and interaction effects of light and water flow on the different growth parameters, taking initial colony size as a covariate. Homogeneity of regression was tested (p> 0.05) to make sure this assumption for ANCOVA was not violated. Post-hoc comparisons were made using the bonferroni correction. Whenever a covariate was found not to be significant, statistical differences were tested using a two-way ANOVA, followed by multiple comparison using the bonferroni correction. The same approach was followed for testing statistical differences in net photosynthetic rate.

3. Results

3.1 Growth

3.1.1 Increase in biomass

All corals grew during the experiment. Increase in buoyant mass and surface area was highest in the high light x high flow treatment. Although buoyant mass and surface area of the coral colonies at the start of this growth period accounted for a significant proportion of the variation in growth ($p<0.005$), a significant interaction between irradiance and water flow was found for both buoyant mass ($p=0.0031$) and surface area ($p<0.0003$) (Table 1, Figure 3).

Table 1: *ANCOVA interaction table giving the main effect of irradiance and flow and the interaction effect between irradiance and flow (IxF) for increase in buoyant mass and surface area between day 125 and day 400. Adjusted means ± S.E. are given for each experimental treatment. N=36. II = intermediate irradiance, LF = low flow HF = high flow, HI= high irradiance.*

	covariate effect	Main effects: Irradiance	Main effects: Flow	Interaction effect: I x F
	p-value	*p-value*	*p-value*	*p-value*
increase in buoyant mass	*0.0018*	*<0.0001*	*<0.0001*	*0.0031*
increase in surface area	*0.0008*	*<0.0001*	*<0.0001*	*0.0003*

light regime		II				HI			
flow rate		LF		HF		LF		HF	
		adj. means	*S.E.*	*adj. means*	*S.E.*	*adj. means*	*S.E.*	*adj. means*	*S.E.*
increase in buoyant mass	*(in mg)*	1058.6	*348.1*	2537.1	*292.2*	2567.5	*318.9*	6108.4	*297.6*
increase in surface area	*(in cm^2)*	3.42	*1.64*	13.38	*1.49*	9.03	*1.54*	31.92	*1.49*

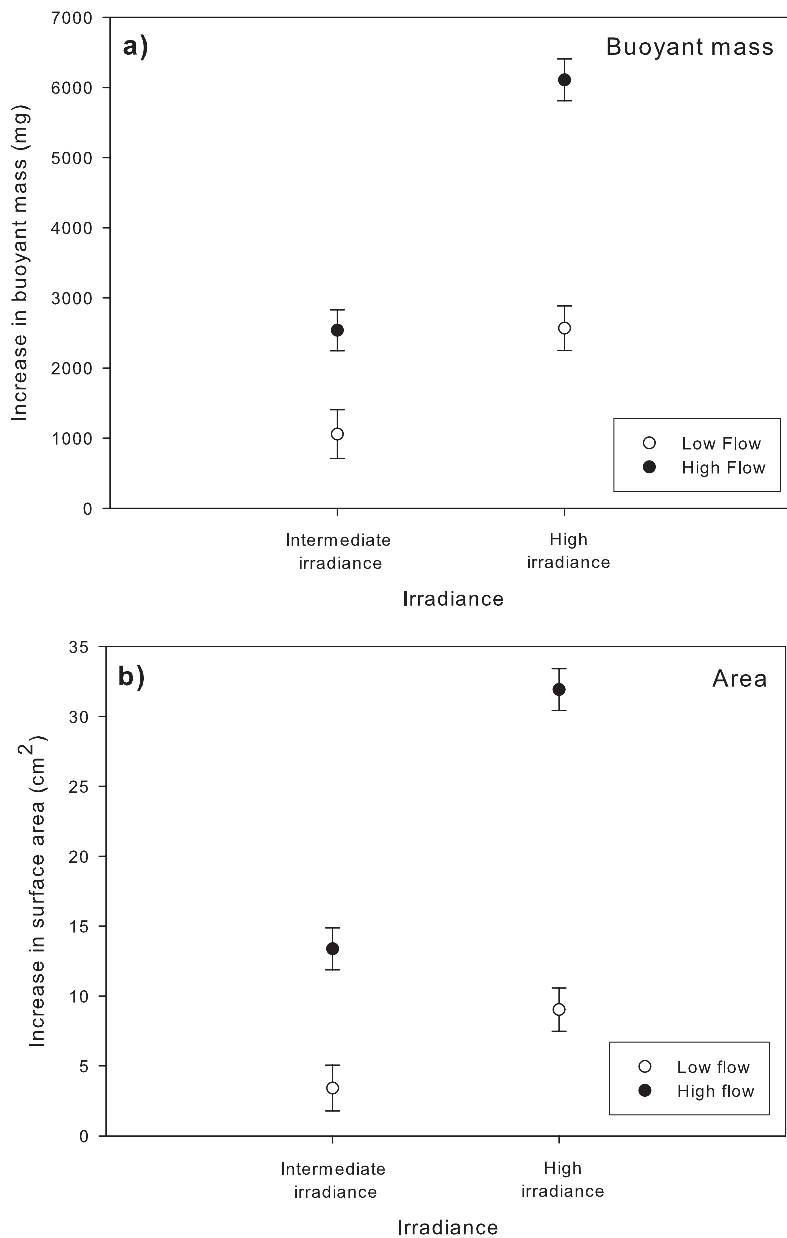

Figure 3: *Average increase in buoyant mass (a) and surface area (b) per treatment during the second growth period. Values are adjusted means ± S.E. N=36 corals (9 per treatment).*

At high irradiance, increasing water flow resulted in a significant increase in both buoyant mass ($p<0.0001$) and surface area ($p<0.0001$). At intermediate irradiance, the effect of increasing water flow also resulted in a significant increase in both buoyant mass ($p=0.017$) and increase in surface area ($p=0.0007$). At low flow, increasing irradiance resulted in a significant increase in buoyant mass ($p=0.041$), but no significant increase in surface area ($p=0.146$). Whereas at high flow, increasing irradiance resulted both in a significant increase in buoyant mass ($p<0.0001$) and surface area ($p<0.0001$) (table 2, Figure 3). No significant difference in increase in biomass was found between high light x low flow and intermediate light x high flow for either buoyant mass ($p=1$) or surface area ($p=0.2912$).

Table 2: *Results of the post hoc comparison test for the effect of light and flow on increase in buoyant mass and increase in surface area. The percentage increase between treatments is also given.*

	increase in buoyant mass		**increase in surface area**	
	p-values	% increase	*p-values*	% increase
effect flow				
at HI	*<0.0001*	**137.91**	*<0.0001*	**253.51**
at II	*0.0172*	**139.66**	*0.0007*	**291.44**
effect irradiance				
at HF	*<0.0001*	**140.76**	*<0.0001*	**138.60**
at LF	*0.0411*	**142.53**	*0.146*	**164.20**

3.1.2 Specific growth rates

Specific growth rates were calculated over the second part of the growth experiment. The buoyant mass of the coral colonies at the start of this growth period accounted for a significant portion of variation in specific growth rate as buoyant mass ($p=0.0120$), while surface area of the coral colonies at the start of this growth period did not significantly influence the specific growth rate as surface area ($p=0.7841$). A significant interaction was detected between irradiance and water flow for specific growth rate of buoyant mass

(p=0.0163). Interaction between irradiance and water flow for specific growth rate of the surface area was detected at a probability level of 9%. (p=0.0884). The main effects of both irradiance and water flow were significant for specific growth rate of the surface area (p<0.0001) (Table 3, Figure 4).

Table 3: *ANCOVA interaction table giving the main effect of irradiance and water flow and the interaction effect between irradiance and water flow (I xF) for specific growth rate of buoyant mass (SGRbm) and specific growth rate of the surface area (SGRarea). Adjusted means ± S.E. are given for each experimental treatment. N=36. II = intermediate irradiance, LF = low flow HF = high flow, HI= high irradiance.*

			Main effects		***Interaction effect***
		covariate effect	**Irradiance**	**Flow**	**I x F**
		p-value	*p-value*	*p-value*	*p-value*
SGR bm	*ANCOVA*	*0.0120*	*<0.0001*	*<0.0001*	*0.0163*
SGR area	*ANCOVA*	*0.7841*	*<0.0001*	*<0.0001*	*0.0960*
	ANOVA	-	*<0.0001*	*<0.0001*	*0.0884*

light regime		**II**				**HI**			
flow rate		**LF**		**HF**		**LF**		**HF**	
		adj. means	*S.E.*	*adj. means*	*S.E.*	*adj. means*	*S.E.*	*adj. means*	*S.E.*
SGR bm	*ANCOVA*	0.0071	*0.0005*	0.0085	*0.0004*	0.0088	*0.0005*	0.0126	*0.0004*
SGR area	*ANCOVA*	0.0067	*0.0005*	0.0100	*0.0004*	0.0087	*0.0004*	0.0135	*0.0004*
	ANOVA	0.0067	*0.0004*	0.0100	*0.0004*	0.0087	*0.0004*	0.0135	*0.0004*

Specific growth rate of buoyant mass was highest in the high irradiance x high flow treatment, compared to all other treatments (p<0.0001). Higher water flow resulted in a significantly higher specific growth rate of buoyant mass at high irradiance (p<0.0001, 43.7% increase), but this increase was not significant at intermediate irradiance (p=0.2987, 19.6% increase). Higher irradiance also resulted in a significantly higher specific growth rate of buoyant mass at high flow (p<0.0001, 48% increase), but this increase was not significant at low flow (p=0.2548, 23.2% increase).

Specific growth rate of the surface area showed a similar picture, although in contrast to specific growth rate of buoyant mass, all differences were significant. Specific growth rate as surface area was highest in the high irradiance x high flow treatment, compared to all other treatments (p<0.0001). Higher water flow resulted in a significant higher specific growth rate of surface at both high irradiance (p<0.0001, 55.6% increase) and intermediate irradiance (p<0.0001, 48.9% increase). Higher irradiance also resulted in a significant higher specific growth rate of the surface area at both high flow (p<0.0001, 34.7%) and low flow (p<0.0084, 28.9%) (Table 4).

No significant difference in specific growth rate is found between high irradiance x low flow and intermediate irradiance x high flow for either buoyant mass (p=1) or surface area (p=0.1784) (not shown in Table).

5

Table 4: *Results of the post hoc comparison test for the effect of irradiance and flow on specific growth rate of buoyant mass (SGR bm) and specific growth rate of the surface area (SGR area). The percentage increase of growth rates is also given.*

	SGRbm		**SGRarea**	
	p-values	% increase	*p-values*	% increase
effect flow				
at HI	*<0.0001*	**43.7**	*<0.0001*	**55.6**
at II	*0.2987*	**19.6**	*<0.0001*	**48.9**
effect irradiance				
at HF	*<0.0001*	**48.0**	*<0.0001*	**34.7**
at LF	*0.2548*	**23.2**	*0.0084*	**28.9**

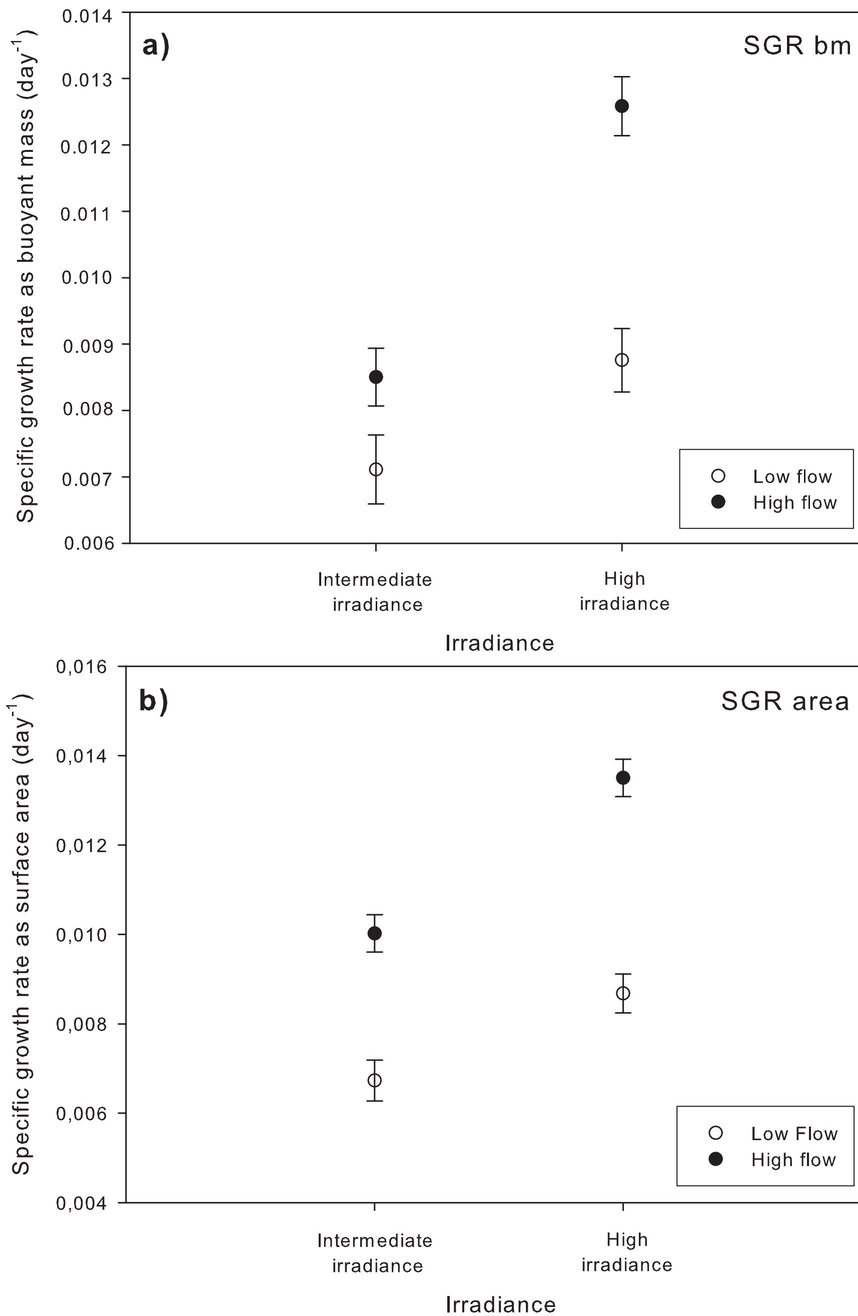

Figure 4: *Interaction plot for the effect of irradiance and water flow on a) specific growth rate of buoyant mass, and b) specific growth rate of the surface area. Values are adjusted means ± S.E.. N=36 corals (9 per treatment).*

3.2 Net Photosynthesis

Since surface area was not a significant covariate for net photosynthesis ($p=0.6212$), a two-way ANOVA was used to test our data for significant differences. No significant interaction is found between the effect of irradiance and water flow on net photosynthesis ($p=0.0573$) and neither the main effect of irradiance ($p=0.8319$) or flow ($p=0.2374$) were significant. None of the specific effects were significant either ($p>0.20$). Average photosynthetic rate at 560 µE m^{-2} s^{-1} was two-fold higher at high flow (0.057 ± 0.011 µmol O_2 cm^{-2} s^{-1}) compared to low flow (0.030 ± 0.003 µmol O_2 cm^{-2} s^{-1}). Average photosynthetic rate at 280 µE m^{-2} s^{-1} was 0.042 ± 0.019 µmol O_2 cm^{-2} s^{-1} at high flow and 0.049 ± 0.014 µmol O_2 cm^{-2} s^{-1} at low flow (Figure 5).

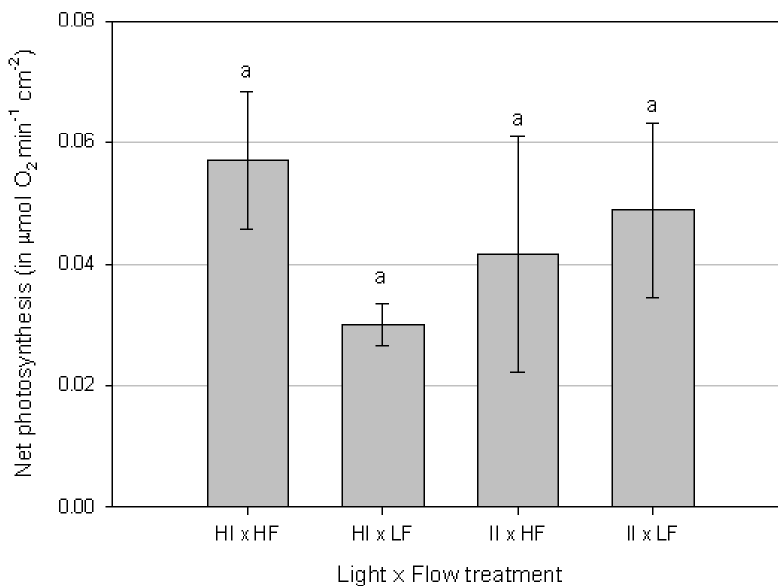

Figure 5: *The interacting effect of irradiance and water flow on net photosynthetic rate at growth irradiance (280 vs 560 µE m^{-2} s^{-1} and flow (5 cm s^{-1} vs 20 cm s^{-1}). See text for details. N=3 corals. Values are mean ± S.D. Means lacking a common superscript differ significantly ($p<0.05$).*

4. Discussion

4.1 Growth

A significant interaction was found between irradiance and water flow for increase in buoyant mass and increase in surface area. Similarly, a significant interaction was found between irradiance and water flow for specific growth rate of buoyant mass. The interaction between irradiance and water flow for specific growth rate of the surface area was not significant at the 5% probability level, but reached significance at a probability level of 9%. Water flow stimulated growth more at high irradiance than at intermediate irradiance, in agreement with our hypothesis. Growth as surface area was consistently much higher than growth as buoyant mass. Since our aquarium system was dominated by crustose coralline algae, this higher rate of surface area growth could be explained by the lack of competition with nuisance/turf algae which allowed the corals to grow more in the periphery.

Interaction between irradiance and water flow was hypothesized to be the result of a limiting effect of low water flow at higher irradiance levels. Indeed, the limiting effect of low water flow becomes obvious when comparing the specific growth rates of corals cultured at high irradiance (600 $\mu E\ m^{-2}\ s^{-1}$) and low flow conditions with intermediate irradiance (300 $\mu E\ m^{-2}\ s^{-1}$) and high flow conditions. Despite having a two-fold difference in irradiance, the specific growth rates at high light x low flow are comparable to the specific growth rates at intermediate light x high flow. Low water flow can limit growth by several mechanisms: 1) limitation of nutrient and/or gas transport (Lesser et al. 1994), and/or 2) inhibition of metabolism as a result of accumulation of oxygen and/ or oxygen radicals, accompanied with light stress (Nakamura et al. 2005; Finelli et al. 2006; Finelli et al. 2007). Light stress reduces coral growth due to energy allocation to (costly) stress responses (Anthony et al. 2002) such as the synthesis of heat shock protein and protecting pigments, 3) reduced photosynthetic rates as a result of oxidative stress (Lesser 1996; Nakamura and Van Woesik 2001; Finelli et al. 2006), potentially impairing light-enhanced calcification, 4) decreased encounter rate of potential food particles (Sebens et al. 1997), and 5) increased

chance for sedimentation (Rogers 1990) and attachment of (and competition with) algae (Smith and Birkeland 2007).

Specific growth rates were lower than expected based on previous studies (49% lower for intermediate irradiance, 45% lower for high irradiance; comparing low flow values) (Schutter et al. 2008). Two reasons can be given to explain this: 1) Specific growth rates were calculated over a different time span (i.e. longer time after nubbing). Since specific growth rates of *G. fascicularis* decrease in time (Schutter et al. 2008), calculating them over a later time span will result in lower specific growth rates. In the current study, specific growth rates were calculated between day 125 and day 400 after nubbing, while Schutter et al. (2008) calculated them between day 1 and day 294 after nubbing. Thus, absolute values should not be compared between these studies. 2) The amount of *Artemia* fed in this study was 16-32 times lower than in Schutter et al. (2008). Heterotrophic feeding increases skeletal growth in an additive way (Ferrier-Pagès et al. 2003). More feeding would therefore probably have resulted in higher growth rates and even more significant results.

4.2 Net Photosynthesis

4.2.1 Net photosynthetic rate and increasing water flow

Increasing water flow did not significantly stimulate net photosynthetic rates at either intermediate or high irradiance. Although the absence of a stimulating effect of increasing water flow on net photosynthetic rate is consistent with previous findings for the effect of flow at 90 µE m^{-2} s^{-1} (**Chapter 2**), this was not expected for higher irradiance levels such as 280 µE m^{-2} s^{-1} and 560 µE m^{-2} s^{-1}. As a result, in this study, differences in skeletal growth rate with increasing flow could not be explained by differences in net photosynthetic rate.

Possibly, the differences in photosynthetic rate were too subtle to be significantly detected using our approach and/or more measurements are needed to detect significant differences between treatments. This might also be the reason why the two-fold difference in photosynthetic rate between high irradiance x low flow and high irradiance x high flow

(0.030 ± 0.003 versus 0.057 ± 0.011 μmol O_2 cm^{-2} min^{-1}) was not significant using a two-way ANOVA. A decreased photosynthetic rate at high irradiance x high flow is plausible, since oxygen accumulation within coral tissues under these conditions is thought to result in photo-inhibition of photosynthesis (Lesser 1996; Nakamura and van Woesik 2001; Finelli et al. 2006). Further research is needed to validate whether increased growth rates with increasing flow at high irradiance are the result of the effect of flow on net photosynthesis.

A second explanation for the differences in skeletal growth is the occurrence of oxidative stress and subsequent need for photo-protection. Photo-protective mechanisms (e.g. Asada 1999; Leggat et al. 1999; Shick and Dunlap 2002) are likely to be energetically costly (Finelli et al. 2006; Hoogenboom et al. 2009). Possibly, more energy is allocated to photo-protective mechanisms in the high irradiance x low flow treatment, resulting in lower growth rates compared to the corals in the high irradiance x high flow treatment. It is therefore likely that increased flow rates lead to increased coral growth at high irradiance levels through the relief of oxidative stress. Possibly, increased heterotrophic feeding (e.g. *Artemia* and/or dissolved organic matter) and/or nutrient uptake with increasing flow (Sebens et al. 1997; Atkinson and Billiger 1992) also played a role. Kaandorp et al. (2005) demonstrated the role of (hydro-dynamically created) gradients of inorganic carbon and nutrients in controlling coral growth and morphology using diffusion–dominated computer models. The importance and contribution of these factors should to be quantified in future experimental research.

4.2.2 Net photosynthetic rate and increasing irradiance

Increasing irradiance also did not significantly stimulate net photosynthetic rates at either low or high flow rates. Net photosynthetic rates could therefore not explain differences in growth with increasing irradiance. This is in contradiction with the light-enhanced calcification hypothesis that assumes that the enhancement of calcification is mediated by photosynthesis (Allemand et al. 2004; Schutter et al. 2008). Schutter et al. (2008) demonstrated a positive relationship between specific growth rate and net photosynthesis for *G. fascicularis*. However, these findings were based on a (short-term) photosynthesis-

irradiance curve, which is possibly different from photosynthetic measurements of corals that are long-term adapted to a certain irradiance. Corals are known to optimize their net photosynthetic rate in order to minimize losses in their metabolic activity (Titlyanov and Titlyanova 2002b). It is therefore possible that the net photosynthetic rates of both the corals at 280 µE m^{-2} s^{-1} and at 560 µE m^{-2} s^{-1} were optimized to similar rates. Moreover, differences in net photosynthetic rate are likely so subtle that significance of measurement is not easily reached.

Besides photosynthesis, the most important factor likely to be co-responsible for increased growth with increasing irradiance is light respiration. Light respiration occurs in the light and is generally much higher than dark respiration due to the presence of photosynthetically produced oxygen and possibly also due to the direct use of translocated photosynthetically fixed carbon. Gross photosynthesis is postulated to be much higher at higher irradiance, but after subtracting the postulated increased light respiration at higher irradiance, the resulting net photosynthesis might be the same (postulated according to Al-Horani et al. 2003a, Figure 6). Light respiration results in a higher availability of metabolic CO_2, which is the major form of dissolved inorganic carbon that is used for calcification (70%, Furla et al. 2000).

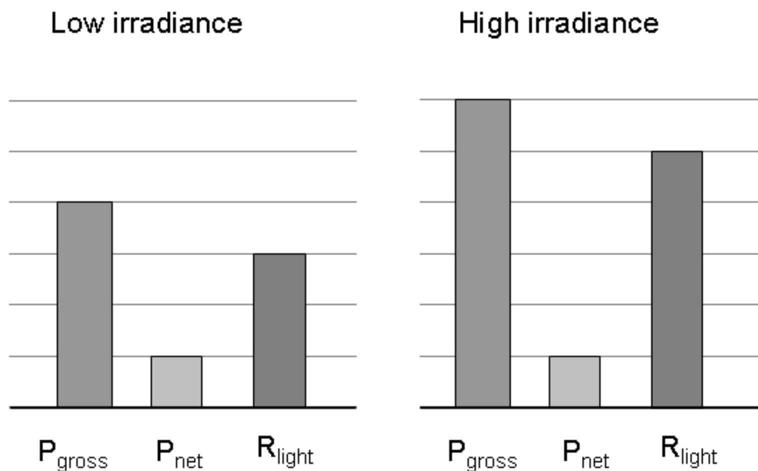

Figure 6: *Postulated effect of irradiance on gross photosynthesis (P_{gross}), light respiration (R_{light}) and net photosynthesis (P_{net}), based on Al-Horani et al. 2003a.*

The phototrophic enhancement of coral growth with increasing irradiance (i.e. light-enhanced calcification) might thus not only be related to increased (net) photosynthesis, but also to light respiration. A limited number of studies on light respiration in corals is known, since it requires sophisticated techniques such as oxygen microsensors (Al-Horani et al. 2003a) or other methods that can separate respiratory fluxes from concurrent photosynthetic fluxes (e.g. oxygen isotopes (^{18}O) (Grande et al. 1991). To the best of our knowledge, the enhancement of light respiration with increasing irradiance has not been studied in scleractinian corals. However, a positive relationship between irradiance and light respiration has been found for several phytoplankton species (Lewitus and Kana 1995).
Nevertheless, differences in light respiration are not likely to explain the interaction between irradiance and water flow. Since photosynthesis is postulated to supply the coral with more oxygen and carbon compounds for respiration than water flow, the enhancing effect of water flow on light respiration is postulated to be small compared to the enhancing effect of irradiance. The most likely mechanism for the interaction between irradiance and water flow is the reduction of oxidative stress and costs associated with photo-protection with increasing water flow.

5. Conclusion

Water flow enhances coral growth both at intermediate and high irradiance. The importance of flow is demonstrated at high irradiance levels, since coral growth at high irradiance x low flow is comparable or even less than coral growth at lower irradiance levels. Thus, water flow is of increasing importance to coral growth with increasing irradiance levels.
The enhancement of coral growth with either increasing flow or increasing irradiance could not be explained by a corresponding change in photosynthetic rate. It is postulated that photosynthetic rates were optimized in each treatment, but that a significant amount of (photosynthetic) energy was allocated to photo-protective mechanisms and the response to oxidative stress in the low flow treatments. Enhancement of coral growth with increasing water flow would therefore be related to the relieve of oxidative stress. The potential effect of light respiration needs further study.

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Chapter 6

The biology and economics of coral growth

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Abstract

To protect natural coral reefs, it is of utmost importance to understand how the growth of the main reef-building organisms - the zooxanthellate scleractinian corals - is controlled. Understanding coral growth is also relevant for coral aquaculture, which is a rapidly developing business. This review paper provides a comprehensive overview of factors that can influence the growth of zooxanthellate scleractinian corals, with particular emphasis on interactions between these factors. Furthermore, the kinetic principles underlying coral growth are discussed. The reviewed information is put into an economic perspective by making an estimation of the costs of coral aquaculture.

Key words: *corals, growth, aquaculture, zooxanthellate Scleractinia*

1 Introduction

Being the main builders of coral reefs, zooxanthellate scleractinian corals (i.e. calcifying corals that live in symbiosis with microalgae – the zooxanthellae) are of crucial importance for marine ecology. In addition, coral reefs represent a high economic value as a source of food (Bryant et al 1998) and natural products (Fusetani 2000), by being an attractive resource for tourism (Bryant et al. 1998) and by forming a natural protection of coastlines. It has been estimated that approximately 10% of the world's population is directly or indirectly depending on coral reefs. However, reefs are currently under high pressure, mainly caused by anthropogenic disturbances such as overfishing, pollution, eutrophication and human-induced climate change (Hughes et al. 2003). Also the trade in aquarium ornamentals has increased in the last decades, and is now also becoming a threat for natural populations of reef organisms including scleractinian corals (Wabnitz et al. 2003; Knittweis et al. 2009). This has resulted in an increased effort to develop cost-effective in situ (sea-based) and ex situ (aquarium) coral aquaculture methods. An example of this is the CORALZOO project, in which scientists and public aquaria collaborated to improve techniques for breeding and husbandry of scleractinian corals (Osinga 2008).

To understand reef development in a changing environment, it is crucial to identify the factors that determine the growth rates of corals and to understand how these factors interact. The same knowledge is needed for efficient breeding of corals ex situ. Furthermore, in this respect, it is important to understand the kinetics of coral growth, which determines how proliferation of biomass develops in time.

This mini-review presents an overview of studies describing effects of environmental factors on coral growth rates. Based on this overview, we will try to explain how coral growth is controlled. Our views will be further supported by new experimental data obtained during the CORALZOO project, which have not been published elsewhere. Secondly, we will discuss the kinetic principles underlying coral growth. Finally, the information will be put into an economic perspective: the costs of coral culture will be analyzed in the view of the biological information provided.

2 The coral growth process

Zooxanthellate Scleractinia represent a true symbiosis. The coral provides shelter and nutrients to the algae, while the algae translocate a substantial proportion of their photosynthetically acquired organic carbon to the coral host. The translocated photosynthetates are used by the host for respiration and biomass buildup (Muscatine and Cernichiari 1969; Muscatine 1990). The coral also acquires organic carbon through feeding on a wide range of particulate and dissolved organic materials (reviewed by Houlbrèque and Ferrier-Pagès 2009). A third important characteristic of scleractinian corals is that they form massive calcium carbonate skeletons through a process called "calcification" (see review by Gattuso et al. 1999). To enable calcification, scleractinian corals synthesize an organic matrix around which calcium carbonate is deposited (Allemand et al. 1998). For a more detailed description of the physiology of zooxanthellate corals, we refer to reviews by Muscatine (1990), Dubinsky and Jokiel (1994), Titlyanov and Titlyanova (2002b) and Furla et al. (2005).

3 Factors influencing coral growth

Taking into account the three major physiological processes described above (photosynthesis, heterotrophic feeding and calcification), the following basic requirements (building blocks) for coral growth can be identified: light, carbon dioxide (CO_2) and inorganic nutrients (needed for photosynthesis); organic food (needed for organic tissue synthesis and organic matrix synthesis); calcium and carbonate ions (Ca^{2+} and CO_3^{2-}, needed for skeleton formation). In addition to these basic requirements, water movement (flow) is an important factor facilitating coral metabolism. Flow enhances the exchange of gases (O_2, CO_2) and dissolved compounds (nutrients, metabolic waste products) between the coral and its environment. Hence, insufficient flow may lead to depletion of resources and/or accumulation of inhibiting substances.

Several other factors have been reported to influence coral growth, either positively or negatively. These factors include temperature and pH (Reynaud et al. 2003; Langdon and

Atkinson 2005; Anthony et al. 2008), iron (Ferrier-Pagès et al. 2001), zinc (Ferrier-Pagès et al. 2005), competition and predation (Fabricius 2005 and references therein), pollution (Jones 2005; Haapkylä et al. 2007; Danovaro et al. 2008), sedimentation (Van Katwijk et al. 1993; Torres 2001, Fabricius 2005), UV radiation (Jokiel and York 1982; Kuffner 2001; Torres et al. 2007), and dissolved oxygen (DO). Despite its key role in metabolism, very few scientists have investigated the potential role of DO as a growth-controlling agent for corals, probably due to the technical complexity of working under low DO concentrations. Rinkevich and Loya (1984) found that aeration of the water significantly enhanced dark calcification in *Stylophora pistillata*. They suggested that under non-aerated conditions, dark calcification in this species was limited by low DO due to the absence of photosynthesis. Fossil records suggests that reductions in DO concentrations were one of the causes of prehistoric mass extinction events of Scleractinia (Van de Schootbrugge et al. 2007). In addition to being a potentially limiting factor, high DO concentrations inside coral tissue are assumed to have a negative effect on coral metabolism (Lesser 1997, Finelli et al. 2006).

In the following subsections, we will more extensively review studies on the primary requirements for coral growth: light, inorganic nutrients, food, dissolved inorganic carbon (DIC, which includes carbon dioxide, bicarbonate and carbonate), calcium and water flow. We will also discuss the role of genetic variability.

3.1 Light

There is no doubt that light plays an important role in the growth of zooxanthellate corals. The coral host is very well adapted to facilitate light capture by its symbiontic algae due to the optimal light reflecting properties of the calcium carbonate skeleton: multiple scattering on coral skeletons enhances light absorption by symbiotic algae (Enriquez et al. 2005). Photon Flux Density (PFD, also known as irradiance) and growth/calcification are often positively correlated (Goreau 1959; Chalker 1981; Marubini et al. 2001; Reynaud et al. 2004; Schlacher et al. 2007; Schutter et al. 2008). Although a direct stimulation of calcification by light was suggested by Al-Horani et al. (2003a), it is important to realize that the corals themselves are mainly indirectly influenced by light, whereas the zooxanthellae can be

directly light-limited. Light-related growth limitation in corals may have three causes: I. Insufficient production of photosynthates (Titlyanov et al 2001). II. Insufficient translocation of photosynthates, for example after enrichment of seawater with inorganic nutrients (Marubini and Davies 1996, see also section on nutrients below). III. A decrease of the internal pH due to lower photosynthesis, leading to less favorable conditions for calcification (Schneider and Erez 2006). In addition, light may become inhibiting at high photon flux densities. After long exposure to high PFD, the increase in maintenance energy required to repair the light-induced damage to the photosystem will exceed the gain in photosynthetic energy, leading to a retarded growth (photoinhibition, Iglesias-Prieto et al. 1992).

The coral-zooxanthellae holobiont adjusts its photosynthetic potential to the prevailing environmental conditions. Such photoacclimation is achieved either by increasing/ decreasing the number of zooxanthellae per cm^2 of coral surface (probably a host-controlled mechanism: adaptive bleaching – Kinzie III et al. 2001; Fautin and Buddemaier 2004) or by adjusting the pigment density (a zooxanthellae-controlled mechanism). Both processes occurred simultaneously within a period of 30 days after transplanting fragments of *Stylophora pistillata* from high to intermediate PFD and from intermediate to low PFD (Titlyanov et al. 2001). In addition, also the pigment composition of the zooxanthellae is variable and adjusted to the available spectrum of light (Dustan 1982).

The in hospite photosynthetic potential of zooxanthellae in corals is usually determined by measuring a Photosynthesis/Irradiance (PI) curve (Figure 1). PI curves can either be obtained using direct assessment of electron transport rates (ETR) as a measure for photosynthesis using Pulse-Amplitude Modulated fluorometry (PAM, e.g. Ulstrup et al. 2006) or indirectly from oxygen evolution measurements. Oxygen-based PI curves provide characteristic numbers such as the compensation point (i.e. the irradiance at which photosynthetic oxygen production equals respiratory oxygen consumption) and the saturation point I_k (the point on the X-axis of the curve where the initial, linear slope of the curve intersects with the horizontal asymptote resembling maximal photosynthesis – see Fig 1. This point is also referred to as Talling index (Barnes and Chalker 1990). Due to photoacclimation, specimen of the same species growing under different light regimes may show different PI curves (Figure 1).

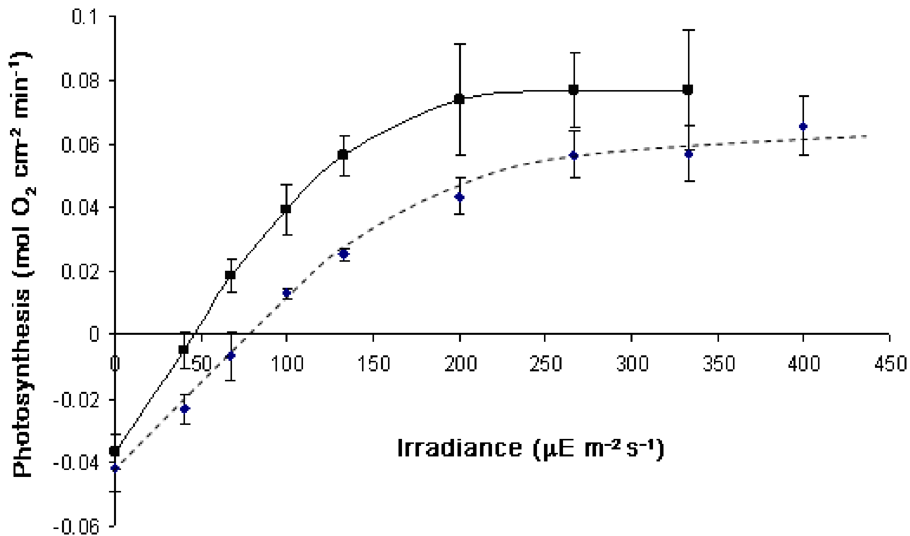

Figure 1: *Photosynthesis/Irradiance curves of* Galaxea fascicularis *based on oxygen evolution measurements (methodology according to Schutter et al. 2008, corals were incubated in 1500 cm³ incubation chambers equipped with a magnetic stirrer). The two curves each represent averages of two groups of four colonies that had been raised under 300 (solid line) and 600 (dotted line), respectively. The Talling index I_k is indicated for both curves.*

Corals raised under low light may exhibit the same rate of net photosynthesis as corals growing under high light. Hence, to assess the PFD at which light becomes limiting, it is better to use a normalized PI curve, based upon measurements done on corals only at their ambient PFD. The saturation point of such a normalized curve represents a species specific saturation point (hereinafter referred to as I_ks), below which photoacclimation cannot longer compensate for the reduced influx of photons. I_ks may vary as a result of variability in other environmental conditions such as the flow regime around the corals and the availability of inorganic nutrients and food (see next subsections).

3.2 Inorganic nutrients

Both partners of the coral-zooxanthellae holobiont need nitrogen (N) and phosphorous (P) as building blocks for synthesis of proteins and other biomass components. Whereas the zooxanthellae can directly take up N and P in their inorganic forms (Dissolved Inorganic Nitrogen – DIN and Dissolved Inorganic Phosphorous - DIP), the coral host acquires its N and P through heterotrophic feeding (see Section 3.4) and via translocated organic substances produced by the zooxanthellae. According to Falkowski et al. (1984), translocated substances can become very low in nitrogen when the zooxanthellae are DIN-limited. They introduced the term “junk food” to describe the low-N organic excretion products of N-limited zooxanthellae: these substances only provide the coral host with metabolic energy, and not with nitrogen-rich building blocks needed for biosynthesis. It was suggested that the coral host expels the majority of this “junk food” as mucus.

Following this “junk food hypothesis”, it seems logic to assume that addition of DIN can promote coral growth. Many authors have reported that addition of DIN promotes zooxanthellae growth and augments the pigment production of the zooxanthellae thus stimulating the overall net photosynthesis rates of the holobiont (Hoegh-Guldberg and Smith 1989; Dubinsky et al. 1990; Stambler et al. 1991, 1994; Marubini and Davies 1996; Marubini and Thake 1999; Ferrier-Pagès et al. 2000, 2001; Grover et al. 2002; Langdon and Atkinson 2005; Tanaka et al. 2007), although the photosynthesis rate per algal cell can decrease due to self-shading effects (Dubinsky et al. 1990). Most of these authors (Stambler et al. 1991, Marubini and Davies 1996, Marubini and Thake 1999; Ferrier-Pagès et al. 2000, 2001; Langdon and Atkinson 2005; Tanaka et al. 2007) also tested the effects of DIN addition on skeletal growth of the corals, which was inhibited by DIN, or (in the case of moderate nitrate enrichment - Tanaka et al. 2007) only slightly elevated. Both forms of DIN applied (nitrate and ammonium) imposed a similar effect on corals (Marubini and Davies 1996). In general, it can be concluded that raising the external DIN concentration above ambient natural concentrations does not promote coral growth. Apparently, coral growth is not limited by DIN under ambient natural DIN concentrations. Grover et al. (2002) suggested an external concentration of ammonium as low as 0.6 µM

to be sufficient for sustaining zooxanthellae growth.

To explain the observed inhibition of skeletal growth by elevated [DIN], it has been suggested that DIN enrichment disrupts the delicate balance between host metabolism and zooxanthellae metabolism that is needed for optimal functioning of the symbiosis (e.g. Marubini and Davies 1996). It is important to note here that the studies describing effects of DIN-enrichment have all been done under relatively high irradiance levels (200 $\mu E\ m^{-2}\ s^{-1}$ and higher), i.e. under conditions where light is not likely to be limiting. DIN addition under low light (i.e. below I_ks) is not expected to have any direct effect on either the zooxanthellae or the coral.

Some studies on nutrient enrichment (eutrophication) on natural coral reefs (see reviews by Dubinsky and Stambler 1996 and Fabricius 2005) confirm the experimental observations described above (e.g. Kinsey and Davies 1979; Tomascik and Sander 1985; Tomascik 1990; Koop et al. 2001). However, other studies showed a positive correlation between eutrophication and coral growth (Meyer and Schultz 1985; Grigg 1995; Bongiorni et al. 2003a,b). The conflicting results can be ascribed to indirect effects of DIN/DIP enrichment. Enrichment will lead to higher concentrations of particulate and dissolved organic matter in the water column, which may enhance coral growth (by providing additional food) in free-floating nurseries (Bongiorni et al. 2003a,b) and in coral reefs subjected to high water movement (Fabricius 2005). High particle loads may inhibit coral growth in more stagnant waters due to increased sedimentation (Genin et al. 1995). Eutrophication also indirectly affects coral growth by stimulating the growth of turf algae that compete for space with corals (Genin et al. 1995; Fabricius 2005).

Most studies describing effects dissolved inorganic phosphate (DIP) on corals show that DIP negatively affects coral growth, in particular when supplied without a corresponding increase in DIN (Snidvongs and Kinzie III 1994; Ferrier-Pagès et al. 2000). The negative effect of elevated DIP may be caused by the formation of poisonous polyphosphate crystals (Simkiss 1964). There is also a record of DIP-limitation in zooxanthellate corals: Steven and Broadbent (1997) found increased growth of *Acropora palifera* after pulsed additions of phosphate, with or without concurrent enrichment in nitrate. A good overview of studies relating to effects of both DIN and DIP is presented by Fabricius (2005).

In addition to DIN and DIP, iron and zinc have been reported as agents that influence coral growth. Iron enrichment can have effects on the coral-zooxanthellae symbiosis that are comparable to DIN enrichment (Ferrier-Pagès et al. 2001). The role of zinc in coral growth and metabolism has been clearly outlined in another paper by Ferrier-Pagès et al (2005). Zinc is an essential structural component of many enzymes, among which carbonic anhydrase (CA). CA is an ubiquitous enzyme in corals; it is involved in the uptake of Dissolved Inorganic Carbon. As such, CA plays a key role in both photosynthesis and calcification and therefore, zinc limitation may limit overall coral growth. Reversely, high zinc concentrations may inhibit coral growth due to the formation of toxic free radicals, which have been reported to inhibit microalgae growth (Sunda 1991).

3.3 Bicarbonate, carbonate, calcium and pH: the aragonite saturation state

In order to calcify, corals need Ca^{2+} and CO_3^{2-}. Ca^{2+} and CO_3^{2-} are commonly referred to as Ω, the aragonite saturation state, which is the temperature dependent solubility product of aragonite (Mucci 1983). Aragonite is the chrystalline form of calcium carbonate produced by corals. Both Ca^{2+} ions and CO_3^{2-} ions are actively concentrated in the calicoblastic fluid (this is a thin liquid layer between the skeleton and the calicoblastic cells, the cellular layer that secretes the organic matrix of the skeleton) to facilitate precipitation of calcium carbonate. Ca^{2+} is actively transported across the calicoblastic membrane into the calicoblastic fluid by a Ca^{2+} dependent ATP-ase, which exchanges Ca^{2+} for H^+ ions (Al Horani et al. 2003). This is a process that consumes metabolic energy (ATP). The mechanisms by which HCO_3^- and/or CO_3^{2-} are transported across the calicoblastic membrane are hitherto unknown. However, by removing protons from the calicoblastic fluid, the pH of the calicoblastic fluid is increased, which shifts the equilibrium between HCO_3^- and CO_3^{2-} in favor of the latter: a pH of 9.28 and an Ω of 25 were measured inside the calicoblastic fluid of Galaxea fascicularis (Al Horani et al. 2003), which is well above reference seawater levels (8.2 and 4, respectively). These measurements were done under simulated daylight conditions. The calicoblastic pH and Ω were not elevated when the corals were incubated in the dark, indicating that light stimulates calcification. Indeed,

calcification is on average three times higher during the day than at night (light-enhanced calcification - Gattuso et al. 1999). The mechanism by which light promotes calcification is most likely a combination of a higher availability of ATP and a higher internal pH inside the coral, which both result from photosynthetic activity of the zooxanthellae.

It is generally agreed that Ω is positively correlated with coral growth (Schneider and Erez 2006; Marubini et al. 2008) and reef growth (Anthony et al. 2008, Jokiel et al. 2008, De'ath et al. 2009). The concentrations of both ionic components of Ω have been reported to influence coral growth in a similar way (for effects of [Ca^{2+}], see Chalker 1976; Gattuso et al. 1998; Marshall and Clode 2004; for effects of [HCO_3^-] and [CO_3^{2-}], see Marubini and Thake 1999; Marubini et al. 2001; Schneider and Erez 2006; Herfort et al. 2008; Marubini et al. 2008). Hence, [Ca^{2+}] and [CO_3^{2-}], are of equal importance in controlling coral growth. Whereas [CO_3^{2-}] may vary due to short-term and long-term changes in ocean pH (Gattuso et al. 1999; Kleypas et al. 1999), [Ca^{2+}] is rather stable in oceanic waters. Therefore, [Ca^{2+}] is not considered as a very relevant factor with respect to the effect of climate change on calcifying organisms. However, in an aquarium situation, where the ratio between water volume and coral volume is orders of magnitude lower than in nature, the concentration of Ca^{2+} can diminish rapidly and should be adequately monitored and controlled.

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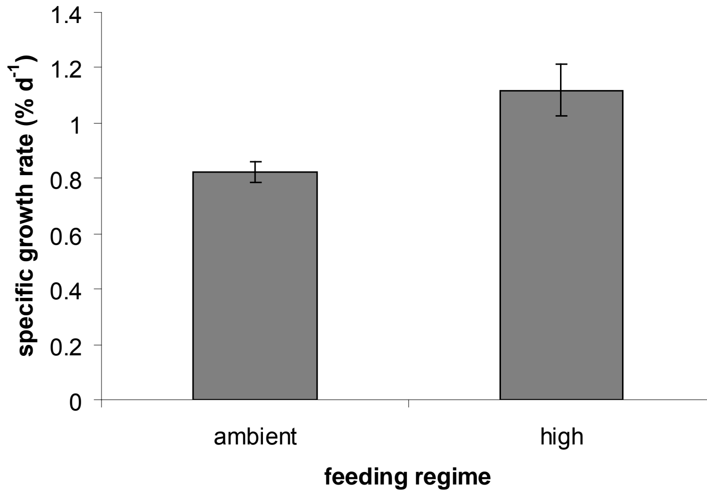
3.4 Food

The heterotrophic feeding biology of zooxantellate corals has recently been reviewed by Houlbrèque and Ferrier-Pagès (2009). Here, we will briefly summarize some important observations on the effects of feeding on coral metabolism and growth.

One of the proposed benefits of feeding is that it supplies the coral holobiont with nitrogen (Dubinsky and Jokiel 1994). In contrast to the effect of DIN addition, which stimulates zooxanthellae, but inhibits growth (see Subsection 3.2), it was shown by Ferrier-Pagès et al. (2003) and Houlbrèque et al. (2003, 2004) that feeding stimulated both zooxanthellae (numbers, pigmentation and photosynthetic activity) and growth of *Stylophora pistillata*. Analogously, we found that high feeding increased both the specific growth rate (Figure 2A) and the photosynthetic capacity (Figure 2B) in colonies of *Seriatopora caliendrum*.



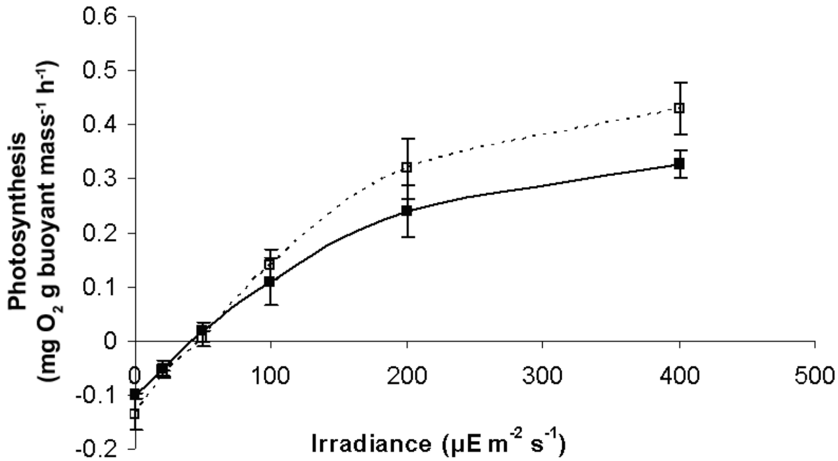


Figure 2A: *Specific growth rates of colonies of Seriatopora caliendrum cultured at ambient aquarium feeding and high feeding (ambient aquarium feeding + 20.000 Artemia nauplii per colony per day). n = 3 for both treatments.* **B.** *Photosynthesis - Irradiance curves for ambient fed colonies (solid line) and highly fed colonies (dotted line). The differences between ambient and high feeding are significant at 100, 200 and 400 μE m^{-2} s^{-1} (paired t-test, n = 3, $p < 0.05$).*

Organic food provides the coral holobiont with nitrogen, carbon and phosphorous in an appropriate biological ratio. Hence, in contrast to enrichment with DIN and/or DIP, providing organic food is not expected to disturb the nutrient balance inside the coral.

The beneficial effects of feeding on growth and photosynthesis appear not to be directly coupled. Food-stimulated photosynthesis is likely to occur only under high light. Both in our study on *Seriatopora caliendrum* and in the study by Houlbrèque et al. (2004) on *Stylophora pistillata*, the stimulating effect of feeding on photosynthesis became apparent only above 200 μE m^{-2} s^{-1}.

In another study that was performed during the CORALZOO project, we found increased growth of *Pocillopora damicornis* as a result of additional feeding, without a concurrent increase in photosynthetic activity (Figure 3A,B). The observed differences in growth could therefore not be attributed to food-induced differences in photosynthetic activity. Either, feeding stimulated the utilization of photosynthetic products by the corals (a food-light interaction leading to a more efficient use of the photosynthetically produced resources), or the effect of feeding was just additive to growth on photosynthetically acquired resources. The possible interrelationships between feeding and photosynthesis will be further discussed in Subsection 4.2.

An aspect of heterotrophic feeding that has often been overlooked is the direct uptake of non-living dissolved organic carbon (DOC) by corals. Sorokin (1973) measured uptake rates of DOC by six common reef-building corals by adding radiolabelled DOC to coral colonies in closed incubation chambers. He found that daily DOC uptake among the six species studied ranged from 13.3 to 29 % of the total amount of carbon present in the coral tissue. Hence, DOC uptake may represent a significant proportion of total food uptake and should not be neglected when estimating a coral's carbon budget. In addition, supply of DOC to corals in culture may be a useful alternative to the commonly used live planktonic or particulate feeds.

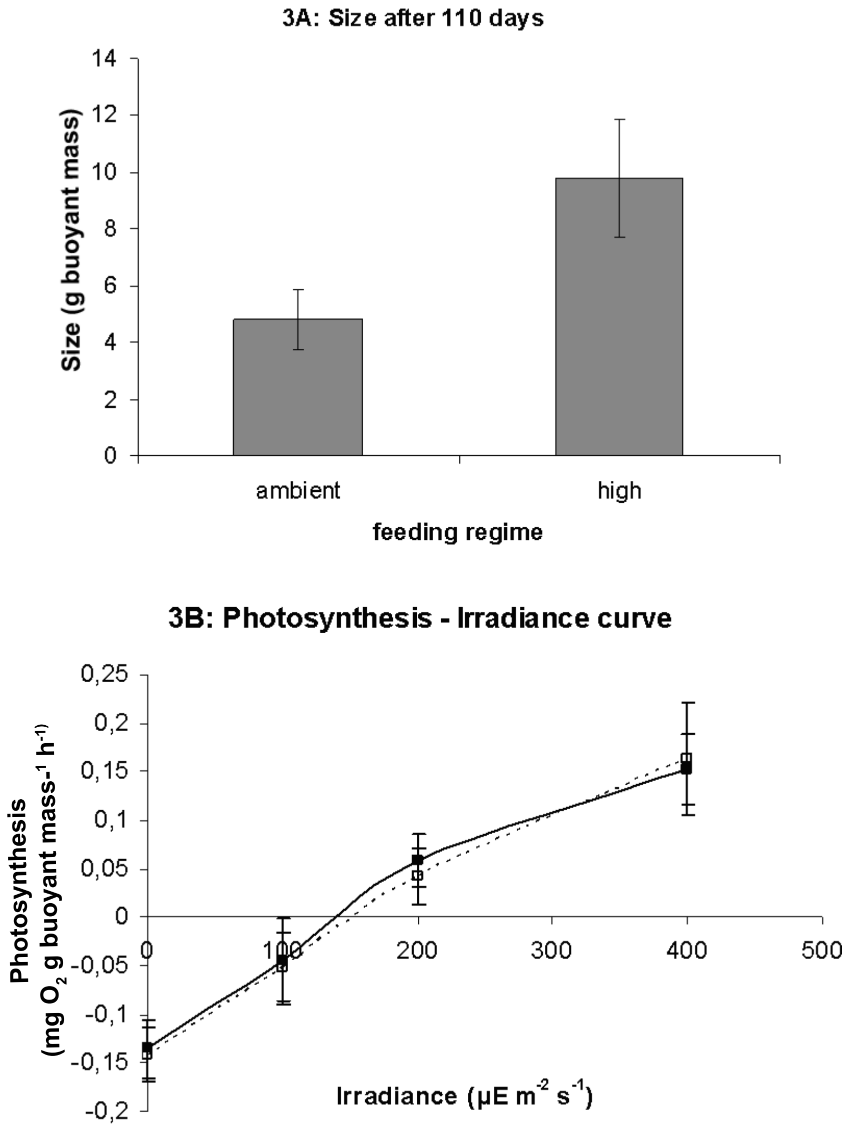


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Figure 3A: *Growth (biomass after 110 days in culture) of nubbins of* Pocillopora damicornis *grown in 40 l aquarium systems under ambient feeding (the food available in the natural seawater used in the aquaria) and high feeding (2000* Artemia *nauplii* l^{-1} *+ 30.000* Tetraselmis suecica *cells* ml^{-1}*). n = 3 for both treatments. Corals were grown at a PFD of 200 µmol photons* m^{-2} s^{-1}*. For details on the methodology, see Lavorano et al. (2008).* **B:** *Photosynthesis – Irradiance curves for colonies of P. damicornis grown under ambient feeding (dotted line) and high feeding (solid line).*

3.5 Water movement (flow)

Water movement (flow) can affect coral growth in different ways. Since corals cannot actively generate water movement, they are dependent on ambient flow for the supply of basic requirements such as oxygen and inorganic carbon (Dennison and Barnes 1988; Lesser et al. 1994), inorganic nutrients (Stambler et al 1991; Atkinson and Bilger 1992; Thomas and Atkinson 1997) and food (Sebens and Johnson 1991; Sebens et al. 1998). Flow-dependent mass transfer of oxygen may explain why Rinkevich and Loya (1984) found aeration-enhanced dark calcification. Second, flow controls the efflux rate of potentially toxic metabolic products such as oxygen and oxygen radicals (Nakamura et al. 2005; Finelli et al. 2006). Third, flow may indirectly promote coral growth by removing sediment and by preventing settlement of fouling organisms such as algae (Fabricius 2005; Box and Mumby 2007). High flow rates may inhibit coral growth. Deformation of the polyps under high flow reduces their prey capture efficiency (Sebens et al. 1997) and will reduce mass transfer of dissolved gases and inorganic nutrients. This may explain why Atkinson et al. (1994) did not find profound effects of water flow velocity on nutrient uptake in flume experiments with *Porites compressa*: they compared relatively low flow rates (~5 cm s^{-1}) with rates exceeding 25 cm s^{-1}. The high rates used by Atkinson et al. (1994) are at the top end of the range that is normally experienced by most corals and may thus have become harmful to the corals.

Experimental data demonstrate that different corals show various responses to changes in flow. Both increased growth (Jokiel 1978; Montebon and Yap 1997; Nakamura and Yamasaki 2005; Chapter 2) and decreased growth (Kuffner 2001) have been reported in relation to increases in flow.

3.6 Genotype

Apart from external factors influencing coral growth, there are also genetic factors that strongly affect the specific growth rate of a genetic individual. Each genet of a particular species has its own specific set of genes and will thus respond differently to different combinations of environmental conditions. Whereas some genotypes will invest more in

growth, others may be better in resisting overgrowth and diseases. Here, we present an experimental example obtained during the CORALZOO project, which shows how genotypic variability affects coral growth. Groups of 10 clones originating from 10 genetically different individuals of *Stylophora pistillata* were grown for 1.5 years under the same conditions in an aquarium. Two out of ten genotypes did not survive in the aquarium. The remaining eight genotypes showed remarkable differences in growth (Figure 4).

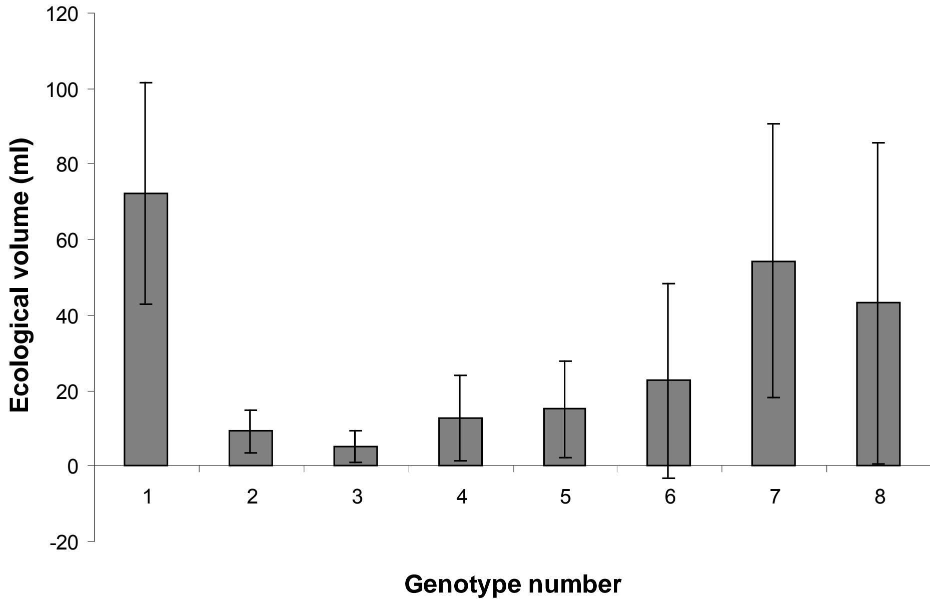

Figure 4: *Biological volumes (determined according to Rinkevich and Loya 1983) of eight genotypes of* Stylophora pistillata *after being in culture for 1.5 years. Error bars represent standard deviations (n = variable, depending on the number of clones that survived). These results are part of a larger study on genotypic variability, which will be described elsewhere.*

This example clearly demonstrates that studies aiming to provide general information on the growth of a species should take into account genetic heterogeneity and present averages obtained from different genotypes. Working with clones obtained from a single genotype only provides information on that particular genotype and cannot be extrapolated to the species level. On the other hand, general physiological mechanisms are best studied using coral fragments that are genetically identical. It remains to be determined to what extent genetic differences in zooxanthellae populations can account for the observed genetic variability.

4 Interactions

Interactions between factors influencing coral growth can be defined as the extent to which one factor increases or decreases the effect of another factor. Many factors described in Section 3 interact, only the most important interactions will be highlighted here.

Light will interact with water flow, because water flow determines both the rate of supply of DIC and inorganic nutrients needed for photosynthesis and the efflux rate of oxygen and oxygen radicals that may inhibit photosynthesis (Finelli et al. 2006). Indeed, a significant interaction between light and flow was found to affect the growth of *Galaxea fascicularis*. A combination of high light and high flow had a stronger positive effect on growth than the individual factors. Hence, a positive correlation between irradiance and growth will be stronger under high-flow conditions (Chapter 5).

Light also interacts with the concentration of bicarbonate (HCO_3^-) in seawater. The positive effect of irradiance on growth is enhanced by adding HCO_3^- (Marubini et al. 2001), which suggests that DIC is limiting coral growth. This is explained by the fact that two of the major physiological processes in corals, calcification and photosynthesis, compete for the same substrate (DIC). Such internal competition for DIC was also suggested by Marubini and Davies (1996) to explain the negative effects of DIN addition on calcification: enhanced photosynthesis resulting from DIN enrichment increases the photosynthetic demand for DIC at the expense of calcification. Marubini and Thake (1999) demonstrated that the inhibiting effects of DIN could indeed be stopped by doubling the concentration of HCO_3^- in the seawater.

Reversely, it has been suggested also that calcification supports photosynthesis by converting HCO_3^- into membrane-permeable CO_2 (the substrate for the photosynthetic key enzyme RuBisco), thus reducing the need for carbonic anhydrase-based carbon concentrating mechanisms (Furla et al. 2005) to supply DIC to the zooxanthellae. This so-called “trans-calcification model” (McConnaughey and Whelan 1997) provides an elegant evolutionary explanation for the massive formation of external skeletons by scleractinian corals. Marshall and Clode (2002) presented data that support this view. These authors used enrichment with Ca^{2+} (the other component of Ω), which stimulated both calcification

and the incorporation of photosynthetically acquired carbon into coral tissue. Increasing [Ca^{2+}] may lower the amount of metabolic energy required for transport of [Ca^{2+}] into the calicoblastic layer and may in this way compensate for the increased metabolic effort to acquire CO_3^{2-} for calcification. However, the trans-calcification model appears to be in contradiction with the suggested internal competition for DIC. Moreover, it is based upon the questionable assumptions that HCO_3^- freely diffuses into the central cavity of the coral (see review by Allemand et al. 2004).

An interesting addition to this discussion is the potential role of heterotrophic feeding in the internal dynamics of DIC, N, P and pH. The reason that feeding gives a similar response of zooxanthellae to enrichment with inorganic nutrient, but without a concurrent decrease in coral growth may be found in the fact that respiration of organic food will simultaneously yield DIN, DIP and DIC in a biologically appropriate ratio. However, since the DIC provided by food digestion is in the form of CO_2, excessive feeding may decrease the pH inside a coral, thus slowing down calcification. Since this effect will be stronger in the absence of light (in the light, the CO_2 produced by respiration will be quickly assimilated by the zooxanthellae), it appears logic to feed aquarium corals during daytime. Indeed, Lavorano et al. (2008) found that daytime feeding of *Pocillopora damicornis* had a stronger positive effect on growth than nocturnal feeding.

4.1 Autotrophy versus heterotrophy: interactions between light and feeding

As reviewed by Houlbrèque and Ferrier-Pagès (2009), there is an ongoing debate on the roles of autotrophy and heterotrophy in zooxanthellate corals. The prevailing views, however, are that heterotrophy provides an alternative for phototrophy (additive effect) and that the balance between phototrophy and heterotrophy is dependent on light conditions. The shifting roles of autotrophy and heterotrophy as described by Anthony and Fabricius (2000) for corals facing increased turbidity and by Grottoli et al. (2006) for bleached corals support this view.

It is very likely that apart from providing an additional source of carbon, nitrogen and phosphorous for both host and symbionts, heterotrophy also supplies several essential components (building blocks) for the biosynthesis of the coral host that it can hardly or not

obtain from translocated photosynthetic products. Such a dependency on heterotrophy for specific components implies that the rate of heterotrophic feeding can actually directly limit coral growth. There are three facts that support this view:

1. There are, to the best of our knowledge, no records of 100% phototrophy in scleractinian corals.
2. Supplementing the water with nutrients stimulates photosynthesis and symbiont density, but does not augment coral growth (Marubini and Davies 1996), even when bicarbonate is added to prevent internal DIC-limitation (Marubini and Thake 1999).
3. The organic component of reef coral biomass is to a large extent of heterotrophic origin (Muscatine and Kaplan 1994, Grottoli 2000). Some components, in particular in the organic matrix of the coral skeleton, appear to be almost exclusively of non-phototrophic origin (e.g. Allemand et al. 1998).

These observations put the observed beneficial effects of feeding (Anthony and Fabricius 2000; Bongiorni et al. 2003a,b; Ferrier-Pagès et al. 2003; Houlbrèque et al. 2003, 2004; Lavorano et al. 2008) on coral growth in a slightly different perspective: feeding does not only provide alternative sources of carbon, nitrogen and phosphorous, it will also augment the utilization of phototrophically acquired resources, leading to a more efficient use of these resources and reduced loss by excretion and mucus production (a lower release of "junk food").

There are two studies that support this view. Houlbrèque et al. (2004) found an increased calcification coinciding with an increased deposition of aspartate when comparing fed colonies of *Stylophora pistillata* to starved colonies and proposed that feeding-induced organic matrix synthesis is determining calcification rates. A second study was done recently by members of our team. The interactive effects of irradiance and food availability on the growth of the branching coral *Pocillopora damicornis* were tested. Nubbins of this species were prepared and cultured as described in Lavorano et al. (2008). Two photon flux densities (100 and 300 $\mu E\ m^{-2}\ s^{-1}$) were tested against two feeding regimes: low, ambient feeding (through the regular supply of fresh natural seawater to the system) versus high feeding, where ambient food was supplemented with a daily batch of freshly hatched nauplii of *Artemia* (starting concentration: 2000 nauplii dm^{-3}). High feeding stimulated growth at the highest PFD, while

no effect of adding food was observed at 100 μE m^{-2} s^{-1} (Fig 5), indicating an interaction between light and feeding.

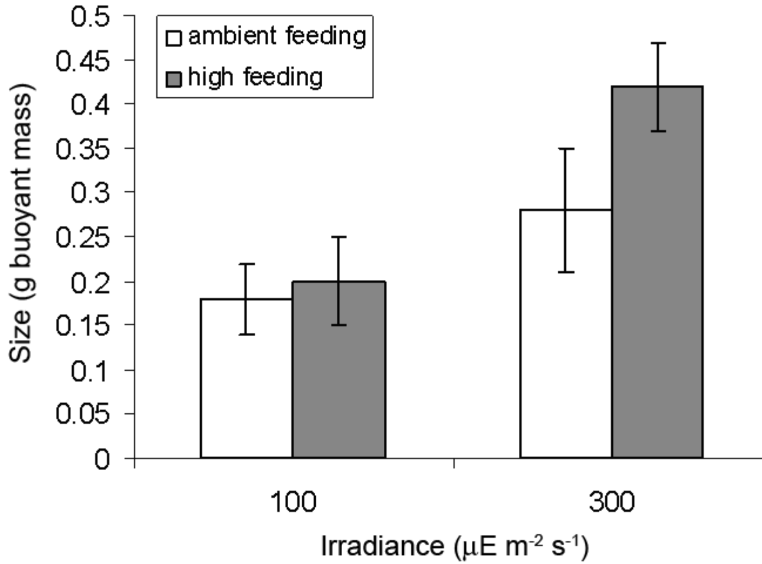

Figure 5: *Interacting effect of irradiance and feeding on the growth of* Pocillopora damicornis*.*

Contrasting with these results, however, is the study by Ferrier-Pagès et al. (2003), who found no interaction between light and feeding on the growth of *Stylophora pistillata*. Their data suggested that the effect of feeding was additive to the effect of light. Ferrier-Pagès et al. (2003) gave no details about the ambient flow velocity in their experimental aquaria, except for the general remark that water motion was generated by an air-stone. Hence, it cannot be excluded that flow-related limitations have occurred under the high light conditions applied in this study, which can mask potential interactive effects between light and feeding.

In general, the uptake of organic food appears to be the most balanced way for a coral to supply itself with a number of resources, including DIN, DIP and DIC for photosynthesis and calcification and essential organic building blocks that cannot be provided by photosynthesis. If food supply is in good balance with light supply and flow velocity, the beneficial effect of feeding on coral growth is likely to be more than just additional to the effect of light.

5 Synopsis: what determines coral growth?

Obviously, there is not one single factor that limits the growth of zooxanthellate Scleractinia. Multiple, interacting factors influence coral growth, which may all become limiting/ inhibiting within their naturally occurring ranges. Furthermore, there is variation among species and within species with respect to growth limitation: genotypic variability is large, different genotypes may have developed different strategies to survive in a fluctuating environment. For example, under a given combination of environmental conditions, light availability may limit the growth of a specific coral individual, but under the same set of environmental conditions, another factor may be limiting the growth of another individual, even when these individuals are conspecifics. This explains why many aquarists report conflicting results when growing individuals of the same species. It also shows that optimization of coral culture is a tedious process, in which many factors should be taken into account. Therefore, large, multi-factorial growth experiments are desired, not only to maximize the productivity for coral aquaculture, but also to further unravel the interactions between potentially limiting and inhibiting factors. Information on interaction may shed new light on the mechanisms that determine coral growth rates.

Despite this complexity, we have attempted to deduce some general mechanisms that may help to explain the phenomena that we observe both in nature and in culture and that may provide guidance for future research. The following working mechanism is proposed for corals growing under natural oceanic conditions in non-stagnant water:

Under a broad range of photon flux densities, the coral-zooxanthellae holobiont is capable to adjust its photosynthetic apparatus in such a way that photosynthesis is always optimal for coral growth (photoacclimation). Below the specific saturation irradiance (I_ks), photoacclimation can no longer compensate for the lower photon flux. Under these circumstances, light availability can limit coral growth due to a reduced input of translocated photosynthetates. Increased heterotrophy can partially compensate for this reduced photosynthetic input. Above I_ks, light is not limiting: the zooxanthellae may become nutrient limited, and the coral host is limited by another factor (e.g. essential food components or DIC). Taking away these limitations will result in a higher growth,

partially caused by a more efficient use of translocated photosynthetates. Crucial in this respect is the role of water movement: when water flow is low or absent, mass transfer of DIC or food may become limiting for coral growth, even below I_ks. In addition, I_ks itself may decrease due to DIC limitation of the zooxanthellae. Furthermore, insufficient water movement will cause inhibition of coral growth under higher irradiance levels, because an increasing proportion of the photosynthetically acquired carbon will then be used for stress responses (i.e. mechanisms to cope with negative effects of accumulated oxygen, oxygen derivatives and metabolic wastes) instead of growth.

6 Growth kinetics

Most studies concerning growth of corals deal with growth rates (see review by Dullo 2005), factors influencing growth (see references in earlier sections) and morphogenesis (Kaandorp and Kubler 2001; Kruszinski et al 2007; Shaish et al. 2006). Only few researchers analyzed the kinetics of coral growth. An elegant and extensive study on the growth of five Caribbean coral species was done by Bak (1976), who reported that the growth of all species studied developed in an exponential way. However, the specific exponential growth rate (i.e. the percentage increase in body mass per unit of time) decreased with increasing colony size. It is important to realize that a growing proportion of the scleractinian coral body mass – the skeleton - is not actively participating to the growth process. Therefore, Bak (1976) related growth to the living surface area (to be precise: to the area covered by the calicoblastic epithelium) and found, by comparing growth rates of small and large colonies, that the rate of calcification per surface area remained unchanged over a long period of time.

Sipkema et al. (2006) presented four hypothetical growth models for sponges, which appear suitable to be applied to corals as well:

1. Linear growth (zero order kinetics), described by:

   $$X_t = X_0 + kt \quad (1)$$

   in which X_t is the size of the coral after time t, X_0 is the size of the coral at t=0 and k is the linear growth rate constant (e.g. in mm day^{-1}).

2. <u>Exponential growth</u> (first order kinetics), described by:

$$X_t = X_0 \cdot e^{\mu t} \qquad (2)$$

in which μ is the specific growth rate constant (e.g. day^{-1}). In this model, the percentage of new biomass formed per day is constant, leading to a J-shaped curve when total body mass is plotted against time.

3. <u>Surface dependent growth</u> (globose organisms)
4. <u>Circumference-dependent growth</u> (encrusting organisms).

By combining the empirical studies on corals by Bak (1976) with the hypothetical growth models for sponges described by Sipkema et al. (2006) we deduced the following basic principles to describe coral growth kinetics:

Branching corals (such as *Madracis mirabilis* in the study by Bak, 1976) proliferate by continuously forming new branches that all have a similar size and shape. Therefore, these corals have a relatively constant surface to volume ratio. Their growth will be appropriately described by first order kinetics (Equation 2), until their size has reached a point where other factors such as gravity-induced forces start to inhibit further growth. A study done in our lab on the branching species *Seriatopora caliendrum*, which was grown under stable, controlled aquarium conditions, showed that the specific growth rate of this species remained constant throughout the monitored period (Figure 6), thus supporting the view that branching species follow first order growth kinetics.

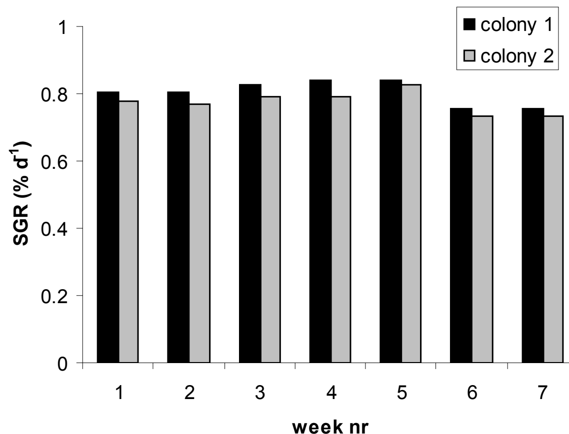

Figure 6: *Specific growth rate (SGR) of two colonies of* Seriatopora caliendrum *during seven consecutive weeks under stable aquarium conditions. Growth was measured as buoyant mass.*

Mass parameters such as wet mass, buoyant mass and dry mass are suitable to monitor growth of these coral species, because they linearly correlate with other commonly used biomass estimators such as surface area, volume, or biological or ecological volume (Rinkevich and Loya 1983).

Boulder-shaped corals and plate-shaped corals follow different growth kinetics. Boulder-shaped corals continuously secrete new layers of calcium carbonate upon their old skeleton, thus continuously increasing the proportion of skeletal mass to total body mass. The living tissue of these corals may either grow with a continuous rate or with a rate that slowly decreases (e.g. *Montastrea annularis* - Bak 1976). In case of continuous tissue growth, total coral growth can best be described by using a surface dependent growth rate constant (Equation 3 in the paper by Sipkema et al. 2006, or similar derivatives for conically shaped objects, etc). Growth of these corals is best determined by using surface area as an estimator for biomass. Plate-shaped corals are most likely to follow circumference-dependent growth kinetics (Equation 4 in the paper by Sipkema et al. 2006, or derivatives thereof). Hence, growth of these corals is best determined by measuring surface area or linear extension rates.

It should be noted that these basic principles only represent a broad generalization. More sophisticated modeling approaches are needed for an exact description of species specific coral growth kinetics. For example, Crabbe (2007) reported that a 3:3 rational polynomial model described the growth of a branching species (*Acropora palmata*) more accurately than the simple first order kinetics model represented by Equation 2. Notwithstanding this, the basic principles outlined above provide a suitable tool to design coral aquaculture systems, as will be discussed in the next section.

7 The economics of coral growth

It has been estimated that economic activities related to corals and coral reefs represent an annual turnover of 375 billion dollars worldwide (Wilkinson 1996, Bryant et al. 1998). The private and home aquarium trade represents a growing proportion of this economic value: the trade in aquarium corals only had an estimated market size of approximately

60 million US$ per year in the period between 1997 and 2001 (Wabnitz et al. 2003), which justifies research efforts focused on optimization of coral aquaculture.

In this section, we present a case study on commercial coral breeding. Daily costs for building and maintenance of coral culture systems (Table 1) were calculated per m^2 culture system surface. Calculation are based upon figures from a coral farm in a public aquarium (Nausicaa, France), where several coral species are being bred for use in public aquaria. The figures presented here concern the branching species *Seriatopora caliendrum*, one of the species that is in culture at the Nausicaa aquarium.

For the commercial aquarium trade, a colony of *S. caliendrum* should have the size of a fist, which corresponds to a wet mass of approximately 100g. A one m^2 aquarium system can host up to 100 colonies of this size.

Table 1: *Overview of costs per category and total operational costs for coral culture systems at Nausicaa aquarium per day. All costs are given in euro's. Systems are depreciated in 10 years.*

Cost category	Low feed scenario	High feed scenario
Manpower	6.53	6.53
Materials	1.42	1.42
Energy	0.22	0.22
Food	0.50	2.00
Depreciation of system	1.10	1.10
Tax and insurance	0.16	0.16
Total costs	***9.93***	***11.43***

We calculated the production costs for 100 g colonies, using fragments that have an initial mass of 10 g as a starting point. Production costs were calculated for two real-life feeding scenario's that had been applied to this coral species (see Figure 3 and its explanation). Annual production of *S. caliendrum* biomass (in kg wet mass) and the corresponding production costs were calculated for both feeding scenarios. The growth of *S. caliendrum* was assumed to be exponential, following first order kinetics (see Section 6: equation 2 and Figure 6). In Figure 5, it is shown that as a result of additional feeding with *Artemia* nauplii, the specific growth rate (μ) for *S. caliendrum* increased from 0.0084 d^{-1} to 0.0115

d^{-1} (i.e. a 35% increase). Both rates were used to calculate the production period, i.e. the time (t) needed for a fragment to grow from a size of 10 g (X_0) to a size of 100 g (X_t):

$$t = \ln (X_t/X_0)\ \mu^{-1} \qquad \text{[days]}$$

The price per colony of 100 g was calculated as follows:

Price (€ $colony^{-1}$) = costs of production system (€ m^{-2} day^{-1}) * culture time (days) / colony density (colonies m^{-2})

It was hereby taken into account that for continuation of the culture, 10% of the harvest is needed as broodstock for the next culture.
Also, the annual production was calculated:

Colonies $year^{-1}$ = production period (days) * 365 (days $year^{-1}$) * number of colonies per production period (colonies)

Since stony corals are a potential resource of natural products with pharmacological properties (e.g. Alam et al. 2001), cultured corals may also be needed as biological materials for drug development studies. For this purpose, the size of the coral individuals harvested is not important. In this particular case, the best strategy for production is to continuously maintain the maximal sustained standing stock (in the example: 100 colonies of 100 g wet mass), and to harvest every day the excess growth (in the example: 0.84 % day^{-1} and 1.15 % day^{-1}). Productivity for *S. caliendrum* was also calculated for this culture approach, hereby again comparing the low-feeding scenario to the high-feeding scenario.
The results of the calculations are summarized in Table 2 and Table 3. This case study shows that optimization can be rewarding: as a result of optimized feeding, productivity increased with 35% while production costs increased with only 15%. The results also show that continuous harvesting reduces the production costs per kg coral considerably.

Table 2: *Production figures for market-size colonies of* Seriatopora caliendrum, *taking into account two production scenarios (low feeding and high feeding). Costs per colony are provided as well as costs per kg, for comparison with the data in Table 3.*

Feeding regime	Production period (days)	Colonies produced per year	Price per colony (€)	Kg coral produced per year	Price per kg (€)
low	274	120	30.20	12	302
high	200	164	25.40	16.4	254

Table 3: *Production figures for continuous production of biomass of* Seriatopora caliendrum *under low feeding and high feeding.*

Feeding regime	Production per year (kg)	Price per kg (€)
low	30.8	118
high	42.2	99

A practical consideration with respect to designing a coral culture in a closed aquarium setting is that semi-continuous production (for example: by renewing every week 2% of the culture) is advantageous over batch production. When operating in a semi-continuous production mode, there will be a constant standing stock inside the culture tank. This will make maintenance easier: feeding regimes and supply of calcium and carbonate do not have to be adjusted continuously to an increasing consumption by a growing standing stock.

8 Conclusions

Due to the adaptive flexibility of corals, their genotypic heterogeneity and the numerous factors that can potentially limit or inhibit coral growth, it is hard to give a clear-cut answer to the question ”What determines coral growth?” The proposed working mechanism described in Section 5 of this paper implies that optimizing coral culture requires a close fine-tuning between light supply, food supply, water movement and DIC concentration. Each species and each genotype will require a different combination of values to maximize

its growth rate. Therefore, efficient high-density coral culture is best achieved by having the individual species and genotypes in separate culture systems.

Acknowledgements

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Chapter 7

General Discussion

Miriam Schutter

1 Introduction

The general aim of this thesis was to study the influence of the abiotic factors light and water flow on the growth and physiology of *Galaxea fascicularis* in closed aquarium systems.

The following objectives were aimed for:

1. to study the effect of water flow on the growth and physiology of *G. fascicularis* (**Chapter 2)**
2. **t**o study the effect of irradiance on growth of *G. fascicularis* and to relate this growth to photosynthetic rate (**Chapter 3**)
3. to study the effect of photoperiod on growth and photo-acclimation of *G. fascicularis* (**Chapter 4**)
4. to study the interaction between light and water flow for growth and physiology of *G. fascicularis* (**Chapter 5**)

In this Chapter, I will highlight the main findings of the thesis work and indicate future directions for research for each factor studied (light, water flow, photoperiod and photoacclimation). Progressing on the review on coral growth and aquaculture in **Chapter 6**, I will discuss how the findings in this thesis can lead to improvement of coral aquaculture and production. Finally, the general conclusions of this thesis will be given.

2 Water flow

2.1 Main findings for water flow

Being sedentary marine invertebrates, scleractinian corals are both dependent on and constrained by the presence of water flow. Water flow affects the exchange rate of dissolved gasses and (in)organic nutrients, prey capture efficiency and removal of sediment and mucus. Some corals have been found to grow more rapidly when flow increases (Jokiel 1978; Montebon and Yap 1997; Nakamura and Yamasaki 2005), showing even an hyperbolic profile of growth with increasing flow rate (Khalesi et al. 2007), while growth rate of other corals was not effected by flow rate (Sebens et al. 2003) or growth even de-

creased with flow rate (Kuffner 2001). However, further research is required because in most studies the water flow rates were not accurately measured, the water flow rate was not sufficiently varied to see an effect or experiments were done for a too short period.
Since zooxanthellate scleractinian corals are symbiotic organisms, relying for a large part on photosynthetic energy from their symbionts, coral growth is expected to be related to the rate of photosynthesis.
In this thesis the effect of flow regime on long-term growth and photosynthesis and respiration rates was measured (**Chapter 2** and **Chapter 5**). The importance of water flow to the growth and well-being of *G. fascicularis* was demonstrated in **Chapter 2**. In the absence of flow, corals grew significantly slower and appeared unhealthy. In the presence of flow (10, 20 and 25 cm s^{-1}), growth rate significantly increased. There was no significant difference in growth rate between the corals grown at 10 cm s^{-1} and 20 cm s^{-1}, while the growth rate of the corals grown at 25 cm s^{-1} was significantly higher. The low specific growth rate in the absence of flow was related to a lower respiration rate. Although the light-enhanced calcification theory proposes that coral growth is enhanced through photosynthesis, this could not be demonstrated. Differences in growth rates at low light intensity (90 µE m^{-2} s^{-1}) between the corals grown at 10 cm s^{-1}, 20 cm s^{-1} and 25 cm s^{-1} could not be explained by either net photosynthetic rates, respiration rates, or the amount of phototrophic carbon left for growth.
Light-enhancement of calcification could either be a qualitative process (i.e. enhancement is related to the presence of light and hence the presence of photosynthesis; i.e. light is needed, but the amount of light is not important - provided that it is not near zero) or a quantitative process (i.e. enhancement is related to the amount of light and hence the rate of photosynthesis). Although the net photosynthetic rate of the corals grown at 10 cm s^{-1} was higher than at 25 cm s^{-1}, the corals grown at 10 cm s^{-1} grew slower. Therefore, in this study, the enhancement of coral growth by photosynthesis is suggested to be a qualitative process.
It was expected that phototrophic feeding and the modulation of photosynthesis by water flow would become more important at high irradiance. Therefore, the interaction between light and water flow was studied in **Chapter 5**. In this Chapter it was demonstrated that

water flow indeed becomes more important for skeletal growth at high irradiance. However, since differences in net photosynthetic rate were not significant, also here mediation by net photosynthesis could no be demonstrated.

Summarizing, there was a positive effect of increasing water flow on growth both at an irradiance of 90 µE m^{-2} s^{-1}(**Chapter 2**), 300 µE m^{-2} s^{-1} and 600 µE m^{-2} s^{-1}(**Chapter 5**). Neither of these differences in growth were supported by a significant increase in net photosynthetic rate, in contrast to our expectations. The positive effect of increasing water flow on coral growth is probably a consequence of both external and internal mechanisms. External mechanisms, such as decreased algal competition and sedimentation, probably allowed the corals to channel more energy to coral growth instead of competition and regeneration (Rinkevich and Loya 1985, Rinkevich 1996). The internal biological mechanisms are not clear yet. Besides photosynthesis, the enhancement of coral growth with increasing water flow could potentially also be related to: 1) increased heterotrophic feeding (i.e. organic nutrient uptake), 2) relief of photo-oxidative stress and therefore reduced energy allocation to expensive photo-protective mechanisms, 3) increased dark respiration (Lesser et al. 1994; Bruno and Edmunds 1998; **Chapter 2**), and/or 4) increased inorganic nutrient uptake such as nitrate or phosphate (Stambler et al. 1991; Atkinson and Bilger 1992; Thomas and Atkinson 1997).

It is suggested that the mechanism of enhancement of coral growth by water flow is different at different irradiance levels. At low and intermediate irradiance, increased growth with increasing water flow might be more related to the increased access to organic carbon sources, possibly to offset a lack of photosynthetic carbon. On the other hand, at high irradiance, the beneficial effect of water flow might be more related to the reduction of photo-oxidative stress through increasing the efflux of oxygen from coral tissue (Finelli et al. 2006). As a consequence, less energy is needed for costly photo-protective mechanisms, permitting higher energy allocation towards growth.

Additionally, the increased supply of (in)organic nutrients with increasing water flow might satisfy the increased need of essential nutrients (e.g. nitrogen and phosphorous) at high irradiance levels.

Since no interaction was found between light and heterotrophic feeding in previous studies (Ferrier-Pagès et al. 2003, Houbrèque and Ferrier-Pagès 2009), the most likely mechanism for the observed interaction between light and water flow is the differential energy allocation due to the relief of oxidative stress with increasing water flow. However, this remains to be confirmed in future studies.

2.2 Future perspectives of water flow

2.2.1 Flow-enhanced calcification

The mechanisms of calcification are still a matter of debate and are being studied at various levels of the coral organism using different approaches. Our holistic approach (measuring calcification as buoyant mass and looking at the coral-algal symbiosis as holobiont) revealed several aspects of coral biology which make it worth to look at the mechanisms behind it:

Carbon balance: It is suggested that, once the inorganic requirements for calcification are met, calcification will depend on the amount of organic carbon available from both phototrophic and heterotrophic sources. More data are required to estimate the total amount of organic carbon that can potentially be channeled towards skeletal growth. In general, but in this instance with respect to flow, carbon balances should be made under the different experimental conditions. For that we should determine:

1. all carbon inputs,
2. amount of photosynthetic carbon that is translocated to the coral host (Muscatine 1984; Dubinsky and Jokiel 1994; Dubinsky and Berman-Frank 2001),
3. amount of carbon that is actually assimilated from the food taken up,
4. carbon losses via mucus production, dark- and light respiration,
5. partitioning of carbon between tissue and skeleton (Anthony et al. 2002).

Light stress: At high irradiance levels, it is proposed that increasing water flow reduces oxidative stress thereby reducing energy allocation towards costly photo-protective mechanisms, resulting in a higher biomass yield on light. This hypothesis could be tested by measuring the energy dissipation rate using Pulse Amplitude Modulated (PAM) fluorometry and the concentration of photo-protective enzymes (e.g. superoxide dismutase) and photo-protective pigments (e.g. xanthophyll pigments) at different flow regimes.

2.2.2 Reduction of experimentation time

Coral growth experiments take a long time and only show overall effects. To understand effects of flow we should in the future make use of shorter mechanistic studies and apply them at a wide range of flows, including unidirectional, bidirectional or oscillatory flow, and turbulent versus laminar flow. These mechanistic studies include:

1) Computer models simulating water flow and boundary layer, and gradients of inorganic carbon and nutrients on different 3D morphologies of corals (e.g. Kaandorp et al. 2003).

2) Measurements of micro-flow patterns, diffusive boundary layers, local absorption of nutrients and gasses and local uptake of food particles to validate the computer models. Sophisticated techniques such as flow microsensors (e.g heated flow thermistor, Labarbera and Vogel 1976; Brand et al. 2007) and microsensors for nutrients (e.g nitrate, De Beer et al. 1997 or dissolved organic carbon, Neudörfer and Meyer-Reil 1997) would be required to measure such parameters experimentally.

2.2.3 Coral morphology

It has been shown that flow affects coral morphology (Chamberlain and Graus 1975; Jokiel 1978; Lesser et al. 1994; Bruno and Edmunds 1998). By adapting its morphology, the coral is able to minimize its diffusional boundary layer thickness and maximize metabolic rates under a variety of flow regimes. For example, the stony coral *Pocillopora damicornis* has a compact growth form under high flow conditions, while its growth form gradually changes into a branching shape when the amount of water flow decreases (Veron and Pichon 1976; Kaandorp et al. 1996). Transplantation of coral colonies from high flow conditions (the outer reef) to low flow conditions (calm bay) is not always successful; the majority of *Pocillopora meandrina* transplanted from the turbulent outer reef into a calm bay died (Maragos 1972). The relationship between coral morphology and coral growth needs further study.

2.2.4 Species

Finally, since the sensitivity to water flow is species-specific (Finelli et al. 2006; Carpenter and Patterson 2007), research on a wide range of species is needed to deduce general rules for optimal flow regimes for the aquaculture of scleractinian corals.

3 Irradiance

3.1 Main findings for irradiance

Light is one of the most important abiotic factors influencing the growth of zooxanthellate scleractinian corals. According to the light-enhanced calcification theory (see Gattuso et al. 1999 and Allemand et al. 1998b for review), calcification of the coral host is enhanced by photosynthesis of zooxanthellae (Goreau and Goreau 1959; Pearse and Muscatine 1971; Allemand et al. 2004). Indeed, on average, calcification in light is about three times higher than calcification in darkness (review by Gattuso et al. 1999). The exact mechanisms of the enhancement of calcification by photosynthesis are still a matter of debate (Gattuso et al. 1999; Furla et al. 2000, Moya et al. 2006, 2008a). According to Moya et al. (2008a), the proposed mechanisms can be classified into two groups,
1) modification of the inorganic chemistry (e.g. carbonate chemistry as site of calcification), and
2) modification of the organic chemistry (e.g. supply of ATP or organic matrix precursors).
The importance of irradiance to coral skeletal growth and the relation with net photosynthesis was studied in **Chapter 3**. It was expected that skeletal growth rate would increase with a higher irradiance because of an increase in net photosynthesis. In **Chapter 5**, the modulating effect of water flow on light use was studied, while the effect of light duration on coral growth was explored in **Chapter 4**.

The positive effect of irradiance on skeletal growth of *G. fascicularis* was demonstrated in both **Chapter 3** and 5: specific growth rate as buoyant mass increased significantly with increasing irradiance. In these studies, the light-enhancement of coral growth was a quantitative process (i.e. related to the amount of light). In contrast, no positive effect of irradiance on skeletal growth was found in **Chapter 4**.
In **Chapter 3**, specific growth rate was related to a photosynthesis-irradiance curve. Both specific growth rate and net photosynthesis increased with irradiance, however, this re-

lationship was not proportional. Net photosynthetic rate increased faster with irradiance than specific growth rate. At high irradiance apparently other factors than light intensity (e.g. availability of bicarbonate or aragonite saturation state, heterotrophic feeding, water flow) became limiting. Further increase of growth at high irradiance levels will only occur when these limitations are taken away (see Figure 1).

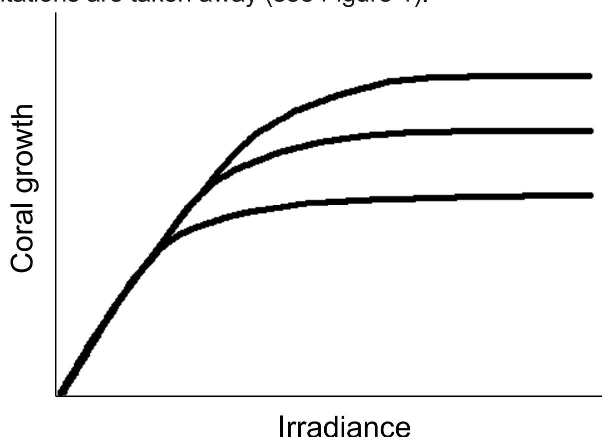

Figure 1: *Conceptual model of how overcoming limitations for calcification can shift the growth-irradiance curve to the right by increasing the light-saturation point.*

Unknown limiting factor

An extreme example of limitations for coral growth was given in **Chapter 4**. In contrast to our results of **Chapter 3**, skeletal growth of *G. fascicularis* did not increase with increasing irradiance (150, 225 and 300 μE m^{-2} s^{-1}). Irradiance was apparently already saturating for growth between 150 and 300 μE m^{-2} s^{-1}. This is consistent with the results of Moya et al. 2006, who found that *Stylophora pistillata* obtained its optimal calcification rate at an irradiance of 100 μE m^{-2} s^{-1}.The factor(s) limiting coral growth could not be conclusively determined in our study.

Aragonite saturation state

Marubini et al. (2001) demonstrated that an increased aragonite saturation state (Ω=2.0 versus Ω=5.0) made no difference for growth at 80 and 150 μE m^{-2} s^{-1}, but that an increased aragonite saturation state did significantly increase growth at an irradiance of 700 μE m^{-2} s^{-1}. This proves the existence of carbonate limitation at higher irradiance levels.

Water flow

In **Chapter 5** it was demonstrated that water flow can relieve certain limitations for resources, or relieve inhibition by harmful substances. A significant interaction between light and water flow was detected: the enhancing effect of light on specific growth rate as buoyant mass was much higher at high flow (48%, $p<0.0001$) than at low flow (23.2%, N.S.), which suggests an effect of flow on light utilization. However, no significant differences were found in net photosynthetic rate, suggesting that enhancement of skeletal growth between 280 and 560 μE $m^{-2} s^{-1}$ was not mediated by net photosynthesis. Water flow could have increased growth by relieving light stress or relieving limitation by (in) organic nutrients.

Heterotrophic feeding

Heterotrophic feeding could be another potential limiting resource for coral growth, as indicated when comparing the studies of Moya et al. 2006 and Houlbrèque et al. 2004 on *Stylophora pistillata*. Moya et al. 2006 found that calcification of *S. pistillata* already obtained its optimal calcification rate at an irradiance level of ca 100 μE $m^{-2} s^{-1}$, while in a separate study Houlbrèque et al. 2004 found that additional heterotrophic feeding could increase the photosynthetic potential of *S. pistillata* at high irradiance levels. This suggests a specific limitation for e.g. N, P or organic nutrients at high irradiance levels and therefore, a similar effect of heterotrophic feeding was expected for calcification (Figure 2) and hence coral growth.

In a preliminary experiment the hypothesis was tested that heterotrophic feeding can overcome growth limitation at high irradiance levels, by measuring differences in short-term calcification (alkalinity anomaly technique, Chisholm and Gattuso 1991) between ambient and enhanced fed corals (n=3) at an irradiance of either 200 or 400 μE $m^{-2} s^{-1}$. Although growth did increase in response to additional feeding, our results in this preliminary study were not conclusive. Suggestions for different approaches are done in section 7.3.2.

Measurement of photosynthesis

We studied the relation between the rate of photosynthesis and growth rate. In future research more attention should be given to the methodology of measurement of photosynthesis. Photosynthesis is measured in short term experiments. When corals are taken out of the culture tank to measure photosynthesis they might either be un-adapted to the new situation (i.e. short-term exposure to different irradiation levels, photosynthesis-irradiance curve, **Chapter 3**) or adapted (i.e. long-term exposure to its growth irradiance, **Chapter 5**). In case the corals are adapted it is expected that results are more reliable.

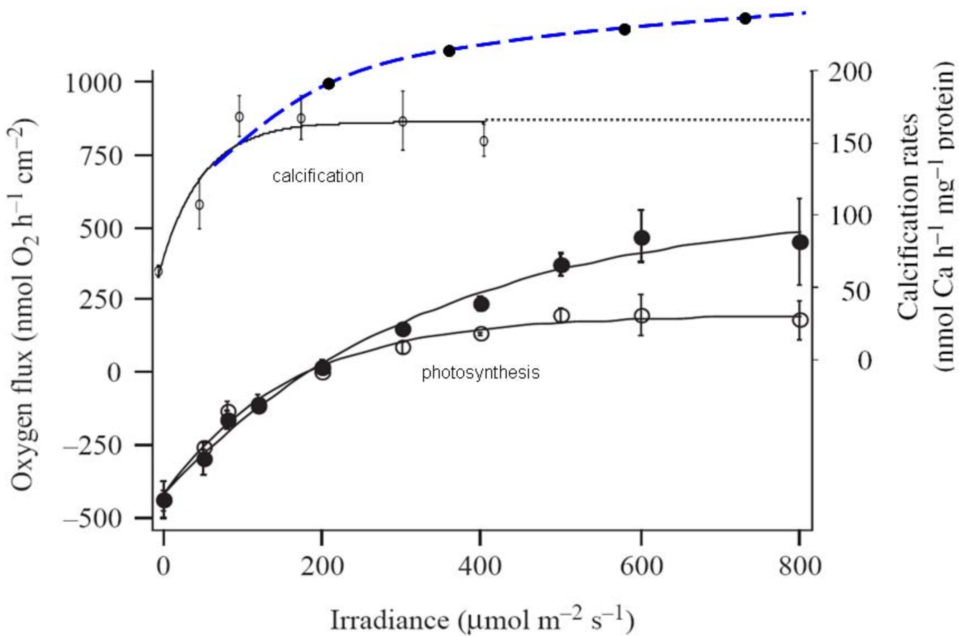

Figure 2: *Comparison of the net photosynthetic rate of the scleractinian coral* S. pistillata *measured under starved (open circles) and fed (closed circles) conditions (Houlbrèque et al. 2004) (n=10, values are means ± S.D.) with the calcification rate of* S. pistillata *measured in a different study under normal feeding conditions (Moya et al. 2006) (n=3). The dotted line is the assumed maximum calcification rate for irradiance levels 400 – 800 µE m^{-2} s^{-1}, while the dashed line (closed circles) is the postulated calcification rate under enhanced feeding conditions.*

Light respiration and mucus production

It is possible that calcification is not directly enhanced by the process of photosynthesis itself, but by increased amount of translocated carbon (Falkowski et al. 1984), increased light respiration (Lewitus and Kana 1995) and/or increased mucus production (Crossland 1987). Further support for the concept that growth is not directly enhanced by photosynthesis, comes from the observation that flow-enhanced photosynthesis is not positively related to skeletal growth at an irradiance of 90 μE m^{-2} s^{-1}(**Chapter 2**). It remains to be determined whether this is true for all irradiance levels.

3.2 Future perspectives for irradiance

3.2.1 Light-enhanced calcification

Since **Chapter 3** and **Chapter 5** gave conflicting results with respect to the enhancement of growth by photosynthesis, another approach is needed to answer the question whether light-enhanced calcification is indeed mediated by net photosynthetic rate, and whether this enhancement occurs throughout the entire light range. Using the holistic approach, creating light-saturation curves for specific growth rate and net photosynthesis, several points of our initial experimental setup can be refined. First, since corals will acclimate to the light intensity at which they are cultured, possibly leading to similar photosynthetic rates at a range of light intensities, photosynthesis of each coral should be measured at its ambient growth irradiance (i.e. each coral is adjusted to its tested irradiance). Second, more data points are needed to draw a solid conclusion. Both the number of irradiance levels at which corals are cultured and the number of corals measured for photosynthesis per irradiance level should be increased to be able to construct reliable light-saturation curves for specific growth rate and net photosynthesis. Also, more refined measurements could be used to detect subtle differences. Third, light-enhanced calcification can be measured more directly by using e.g. the alkalinity anomaly technique at a certain irradiance. The calculation of specific growth rate based on buoyant masses also incorporates lower calcification rates during the night, which results in lower estimates of coral growth than the direct measurement of calcification (i.e. the deposition of calcium carbonate) under a certain light regime.

Ideally, the corals would be cultured in flow-though respirometric cells in which metabolism (photosynthesis and calcification) could be measured throughout a (short) growth period without disturbing the coral. In such a way, a direct correlation between photosynthesis and its effect on deposition of calcium carbonate could be established, providing more insight into the mechanism of calcification. Fourth, we would like to measure light respiration. If the rate of light respiration increases, a larger amount of metabolic CO_2 as source of carbon for calcification is available. The potential acidifying effect of increased metabolic CO_2 is neutralized simultaneously by the secretion of OH^- during photosynthesis (Allemand et al. 2004) and the conversion of CO_2 to HCO_3^- by carbonic anhydrase (Moya et al. 2008b).

3.2.2 Importance of phototrophic feeding versus heterotrophic feeding

The relative importance of phototrophic versus heterotrophic feeding remains poorly understood (Houlbrèque and Ferrier-Pagès 2009). For the work in this thesis it was assumed that phototrophic feeding is of key importance for growth of zooxanthellate scleractinian corals, while under natural, oligotrophic circumstances the importance of heterotrophic feeding lies in the supply of potentially limiting nutrients, such as nitrogen, phosphorus and aspartate (i.e. a more qualitative contribution).

Facts in support of this view are:

a. Heterotrophic feeding is considered to be essential for the supply of potentially limiting nutrients, such as nitrogen, phosphorus and aspartate (Allemand et al. 1998a, Houlbrèque and Ferrier-Pagès 2009). Photosynthates are considered as junk food, since they are generally deficient in nitrogen and phosphorus. Supplementing the water with inorganic nutrients stimulates photosynthesis and zooxanthellae density, but does not augment coral growth (Marubini and Davies 1996), indicating dependency on heterotrophic feeding for specific components.
b. The ability to change trophic mode seems to be a mechanism for sustaining a positive energy balance under less beneficial conditions. Heterotrophic feeding can therefore become quantitatively important under conditions where phototrophic input is reduced, e.g. with depth (Palardy et al. 2006), bleaching (Grottoli et al. 2006) and turbidity (Anthony and Fabricius 2000).

c. Since the influence of heterotrophic feeding on coral growth under different light conditions is merely additive (Ferrier-Pagès et al. 2003, Houlbrèque and Ferrier-Pagès 2009), it seems that once basic qualitative requirements for coral growth are met, increased growth is mostly due to the increased availability of carbon from either a heterotrophic or phototrophic food source.

The quantitative importance of heterotrophic feeding to coral growth might be underestimated in aquaria, since usually more emphasis is put on light requirements. In aquaria increased heterotrophic feeding is unwanted because it puts increased demands on filtration in order to maintain proper water quality.

To test whether enhanced feeding has an additive or interactive effect on coral growth, the following should be taken into account. In our preliminary study, the expected (interactive) effect of heterotrophic feeding on coral growth (i.e. relieving growth-limitation at high irradiance levels) was not evident between 200 μE m^{-2} s^{-1} and 400 μE m^{-2} s^{-1}. However, it is possible that the effect of heterotrophic feeding takes place between different irradiance levels. It is suggested to construct light-saturation curves for calcification of both ambient and enhanced fed corals (see Figure 2) by measuring calcification at a range of (growth) irradiance levels. Using such curves, it would be possible to test whether the point of light-saturation for calcification increases with increased heterotrophic feeding.

The question how heterotrophic feeding increases coral growth also awaits further investigation. The qualitative or “nutrient”-hypothesis, i.e. that heterotrophic feeding stimulates coral growth through an increased supply of N, P and organic nutrients, can be further split up in the following two hypotheses. First, heterotrophic feeding enhances coral growth directly by supplying precursors for the organic matrix (e.g. aspartic acid). Second, heterotrophic feeding enhances coral growth indirectly by increasing photosynthesis and the translocation of photosynthates of a higher quality. The first hypothesis can be tested by adding different amounts of [14C] labeled aspartate (Allemand et al. 1998a) and relating the amount of [14C] labeled aspartate that is incorporated under different feeding conditions to coral growth. Although it has been demonstrated that supply of aspartic acid through heterotrophic feeding is needed for organic matrix synthesis, it is not known if the supply of aspartic acid is limiting under normal conditions. The second

hypothesis could be tested by analyzing the quality of translocated photosynthates of either starved or fed corals.

The quantitative or “carbon”-hypothesis, i.e. that heterotrophic feeding stimulates coral growth through the increased availability of (heterotrophically acquired) carbon, can be split up in the following hypotheses. First, heterotrophic feeding enhances coral growth by providing more building blocks for coral growth. Second, heterotrophic feeding enhances coral growth by increasing respiration and therefore the supply of metabolic CO_2 for calcification. The first hypothesis could be tested using radio active labeled food (e.g. *Artemia*) that can be traced back in coral biomass. The second hypothesis could be answered by measuring isotopes of coral skeleton, host tissue and zooxanthellae. The carbon isotopic value ($\delta^{13}C$) is diagnostic for the origin of carbon, either from heterotrophically acquired fixed carbon or from photosynthetically fixed carbon (Muscatine et al. 1989; Risk et al. 1994; Reynaud et al. 2002; Grotolli 2004). The relative contribution of phototrophic and heterotrophic components to the calcification process can be estimated from skeletal $\delta^{13}C$ and $\delta^{18}O$ values by applying a model of kinetic versus metabolic isotope fractionation (McConnaughey et al. 1997; Heikoop et al. 2000; Maier et al. 2003, Kaandorp et al 2005).

In conclusion, depending on whether heterotrophic feeding is an additional source of carbon or a source of essential nutrients, increased heterotrophic feeding will influence growth in a different way. In the first case, if all other requirements are met, more carbon will result in more growth at all irradiances applied, i.e. in an additive way (Figure 3, dotted line, a). In the second case, growth will increase at higher irradiance levels when essential nutrients become limiting (Figure 3, dashed line, b), i.e. in an interactive way.

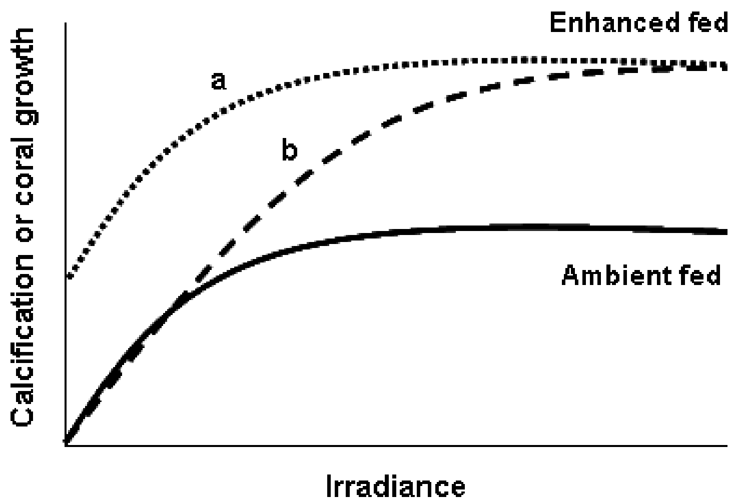

Figure 3: *The relationship between irradiance and calcification under ambient feeding conditions, and the postulated relationship between irradiance and calcification under enhanced feeding conditions. In scenario a, heterotrophic feeding is mainly a source of carbon (dotted line, a), while in scenario b, heterotrophic feeding is mainly a source of essential nutrients (dashed line, b).*

4 Photo-acclimation and photoperiod

4.1 Main findings for photo-acclimation and photoperiod

Although the exact mechanisms of light-enhanced calcification are still a matter of debate (Gattuso et al. 1999; Furla et al. 2000; Allemand et al. 2004; Moya et al. 2006, 2008a), the importance of light for coral growth is beyond doubt. However, the quantity of light that is available for the zooxanthellae is not only determined by the irradiance (i.e. photon flux density), but also by the length of the photoperiod. If the enhancing effect of light on coral growth is related only to the amount of photons (i.e. light flux) received per day, then it can be expected that 1) increasing irradiance will result in increased daily growth rates, 2) increasing photoperiod will result in increased daily growth rates, 3) increasing total light flux will result in increased daily growth rates, and that 4) there will be no difference in

daily growth rate between a total amount of photons applied either in a short photoperiod with high irradiance or a long photoperiod with low irradiance.

In **Chapter 4**, it became obvious that the availability of photons alone cannot enhance coral growth. In contrast to our findings in **Chapter 3**, no positive correlation between light availability and growth was observed, neither with increasing photoperiod, nor with increasing irradiance. This indicated that light was probably in excess. *G. fascicularis* could not be grown under continuous illumination (24 hours light per day). The experimental colonies started bleaching immediately after changing the light duration and died after 14 weeks. In contrast, despite receiving excess light, corals grown under a 16 hour/ day light regime managed to retain growth rates comparable to corals grown with 8 hours light per day. Photo-inhibition of growth was possibly prevented by self-shading resulting in a reduction of the hourly photosynthetic rates. As a result, daily net photosynthesis was not significantly different between the 8 hour day^{-1} and 16 hour day^{-1} light regimes, which is in agreement with their specific growth rates. Since the daily P/R ratio was significantly higher for the corals in the 16 hour day^{-1} light regime, it is suggested that either calcification in this study is primarily mediated by the modification of inorganic chemistry by photosynthesis, since additional photosynthetic carbon does not seem to contribute to additional growth, or that the additional photosynthetic carbon is allocated towards photo-protective mechanisms, explaining the absence of increased growth with increasing availability of photosynthetic carbon.

In line with this thinking, it can be (hypothetically) argued why in this experiment, corals grown under 300 µE m^{-2} s^{-1} did not manage to retain growth rates comparable to corals grown under 150 µE m^{-2} s^{-1}. The specific growth rate of the corals in the 300 µE m^{-2} s^{-1} light treatment was significantly reduced compared to the corals in the 150 µE m^{-2} s^{-1} light treatment. Although there are no photosynthetic measurements of the corals in these treatments, based on the findings in **Chapter 5** it can be assumed that photosynthetic rates were optimized under each irradiance level and were not significantly different from each other. As a result, it is possible that the daily P/R ratio would not have been different between the 150 and 300 µE m^{-2} s^{-1} light treatment. Since the corals in the 300 µE m^{-2} s^{-1} light

treatment were probably stressed by receiving excess light, more (photosynthetic) carbon has been allocated towards photo-protective mechanisms instead of skeletal growth, explaining the reduced growth at 300 µE m^{-2} s^{-1}. Thus, despite receiving the same light flux (8.64 E m^{-2} day^{-1}), the corals in the 16 hour light treatment responded differently to light stress than the corals in the 300 µE m^{-2} s^{-1} light treatment. The different response in specific growth rate as buoyant mass might therefore be explained by a higher daily P/R ratio and a longer period of light-enhanced calcification of the corals in the 16 hour light treatment. However, this remains to be confirmed in future studies.

The mechanisms of photoacclimation are likely to be different under light-saturating and light-limiting conditions. Under light-limiting conditions, light capture and photosynthetic rates will be optimized by adjusting morphological, physiological and/or biochemical parameters of both the coral and its zooxanthellae to increase the harvest and assimilation of light (Titlyanov and Titlyanova 2002b). However, under light-saturating conditions, corals will attempt to reduce their light absorption through e.g. the reduction of zooxanthellae densities and/or photosynthetic pigments (adaptive bleaching – Kinzie et al. 2001, Fautin and Buddermeier 2004) and/or self-shading (Titlyanov 2000, Anthony et al. 2005, Brown et al. 2002). Additionally, protection from excess light will be accomplished through light-protective mechanisms such as the production of photo-protective pigments (e.g. MAA's and xanthofylls) (Titlyanov et al. 2002). This type of photoacclimation can be considered as a stress response.

4.2 Future perspectives for photo-acclimation and photoperiod

4.2.1 Photoacclimation

The mechanisms of photoacclimation to prolonged light duration under light-limited conditions might be completely different from the responses observed in **Chapter 4**. In contrast to their response to light-saturating conditions, corals will attempt to increase their light capture and subsequently their photosynthetic rate under light-limited conditions. Therefore, photosynthetic rates are not expected to change or decrease with increased light duration. As a result, a doubling in light duration might result in a doubling of the daily

amount of photosynthesis and calcification, since it will allow the coral more hours to photosynthesize and to calcify at a higher rate than in the dark (light: dark = 3:1). Under light-limited conditions, it remains to be tested whether the rates of photosynthesis and calcification will remain unchanged with increased light duration and whether they will remain stable during this whole period. Additionally, it remains to be tested whether the daily P/R ratio will be increased, since dark respiration may change in response to a higher availability of photosynthetic carbon. Photosynthesis-irradiance curves could be used as an instrument to determine different aspects of photoacclimation to prolonged light duration, e.g. by determining the light saturation constant (I_k), the compensation intensity (I_c) or efficiency of light use (α).

4.2.2 Growth

If the influence of light on coral growth is all a matter of available photons, then increasing either irradiance, photoperiod or total light flux will all have a positive effect on coral growth. However, since calcification has a hyperbolic relationship with irradiance (Chalker 1981), it is arguable that increasing the light duration will be more beneficial for daily calcification than increasing irradiance (see Figure 4 and previous section). In other words: with a doubling in light duration (2xh), daily photosynthesis and/or calcification are expected to double as well (2xP or 2xC). A doubling in irradiance (2xI) may have a less pronounced effect on P and C, the magnitude of the effect being dependent on the position within the photosynthesis-irradiance curve (Figure 4).

Adding to this, it can be assumed that daily growth rate will be higher when photons are supplied over a long period at a low irradiance than when the same amount of photons is supplied over a short period at a high irradiance. However, such a difference would only be expected if only the high irradiance level exceeds the saturation value I_k, which is the irradiance level at which the initial slope of the curve intersects the horizontal asymptote. In such case, calcification and irradiance are not directly proportional anymore, and a doubling in irradiance will not result in an exact doubling of calcification.

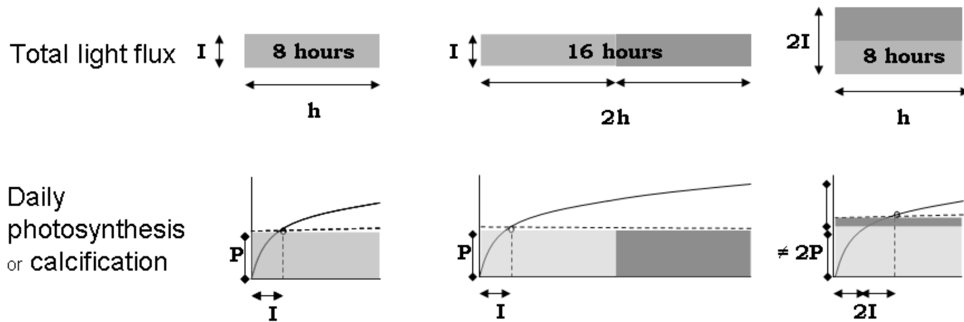

Figure 4: *Schematic overview showing the hypothesized difference between either doubling irradiance or doubling photoperiod (resulting in equal total light flux) on daily photosynthesis or calcification.*

Therefore, it is hypothesized that, under light-limited conditions, a two-fold increase in photoperiod is more beneficial to coral growth than a two-fold increase in irradiance. However, the maximum beneficial photoperiod remains to be investigated. It is hypothesized that a longer light duration and consequently more hours of light-enhanced calcification will increase daily growth rates, provided that there is enough time (i.e. dark period) for repair of photo-damage and provided that normal division synchrony of coral cells and zooxanthellae are not disturbed. Analogously to my previous considerations, external factors such as water flow, heterotrophic feeding and aragonite saturation state will play a role in determining the magnitude of the effect of photoperiod on coral growth.

5 The biology and economics of coral growth

5.1 External and internal factors influencing growth

As became obvious throughout this thesis, there are many factors involved in determining the growth rate of stony corals. In the case of environmental factors, the magnitude of the effect of one factor often depends on the other (e.g. in the case of light and water flow, **Chapter 5**) and each factor may therefore either be limiting, saturating or inhibiting, de-

pending on the level of other factors. **Chapter 6** gives an overview of important external factors that can limit or inhibit coral growth and points out interactions between factors. Besides external environmental factors, also internal genetic factors affect the growth rate of an individual. Each genet will respond differently to a different set of environmental factors (**Chapter 6**; Shai Shafir, pers. comm.).

As mentioned before in this thesis, there is a lack of scientific studies focusing on optimizing coral husbandry. Throughout this thesis, a trade-off has been made between relevance for fundamental coral science and applicability in coral husbandry, which was the main aim of the CORALZOO project.

5.1.1 Relevance for fundamental coral science

By using genetically identical nubbins of *G. fascicularis* we were able to deduce general mechanisms of coral growth. General physiological mechanisms are best studied using coral colonies of a single genotype, since this will leave out variability due to genetic differences. Besides, when using a single genotype, less coral colonies will be required to obtain statistical significant results. As a consequence, less aquarium space and less time and effort for maintaining and analyzing all coral colonies is needed. Additionally, coral nubbins of *G. fascicularis* are easy to create, since their large polyps (average Ø 4mm, in this study) can be easily cut apart using a knife and tweezers. Survival of these nubbins is usually nearly 100%, which makes *G. fascicularis* very suitable as a model species for laboratory studies. Nevertheless, *G. fascicularis* is not the ideal coral species to study coral growth mechanisms. First, its slow growth rate resulted in very long experimental times (up to a year) if we wanted to detect significant differences between treatments. Second, its specific experimental growth rate was not constant in time (and thus dependent on size), making comparisons between sizes difficult.

Based on the data in this thesis and on literature data, the following universal mechanism for coral growth is proposed: the coral-algal symbiosis will adapt to the prevailing light regime by optimizing light capture and light processing at different levels of the organism (Titlyanov and Titlyanova 2002ab), in an attempt to maximize light use. Depending on external environmental conditions, photoacclimation may not always be sufficient to obtain

light saturation for coral growth. Under light-limited conditions, the coral will try to compensate for its reduced phototrophic input by heterotrophic feeding or by reducing potential losses (e.g. by lowering respiration and/or mucus production). Under light-saturating conditions, other environmental factors start to limit or inhibit coral growth, e.g. inorganic nutrients, heterotrophic food, inorganic carbon or the presence of water flow. Water flow is very important in both situations, since without flow it is difficult to adapt to both a heterotrophic life style (no supply of food) and to a phototrophic life style (light stress due to lack of removal of inhibiting substances produced during photosynthesis).

The scientific approach would benefit from multi-factorial experiments (e.g. **Chapter 5**), since such experiments will provide more insight in the interactions between factors. Such insight will provide indications on mechanisms and might thus serve as guidelines for mechanistic studies. The potential disadvantage of the multi-factorial approach is the requirement of more coral colonies, more aquarium space, more time for monitoring and maintenance. However, although single-factor experiments require less space and time, such experiments only allow evaluation of the factor under study under the conditions of the experiment, thus yielding much less information. Ultimately, multi-factorial experiments will produce more information while using less coral colonies, especially when using statistical designs.

Besides using multi-factorial growth experiments, experiments could be improved by shortening experimental time (e.g. by using short term measurements) and, depending on the question asked, choosing more specific measurements of coral growth (e.g. calcium carbonate deposition or organic matrix synthesis). As explained in section 6.3.2., the mechanism of light-enhanced calcification can be studied more precisely by measuring calcification instead of coral growth. Specific, but destructive methods such as measuring the incorporation of radioisotopes into the skeleton (Tambutté et al. 1995, 1996) or organic matrix (Allemand et al. 1998a) can be used. However, in such case, only a single set of experimental conditions can be studied per incubation. If one wants to study changes in growth over time and/or under different experimental conditions (e.g. constructing a light saturation curve for skeletal growth or calcification), non-destructive measurements of coral growth should be used. Examples of non destructive measurements are the

buoyant weighing technique for skeletal growth (Jokiel 1978, Davies 1989) or the alkalinity anomaly technique for calcification (Chisholm and Gattuso 1991). Downside of using more specific measurement of coral growth is that coral growth parameters such as ^{14}Ca incorporation into the skeleton are not easily extrapolated to relevant biomass parameter for coral culture.

5.1.2 Relevance and applicability in coral husbandry

The relevance of our experiments for aquarium practices lies in the long-term monitoring of coral growth and measuring a biomass parameter that is relevant for aquaculture (i.e. the experiments approximate real-life coral production scenarios). Measurement of growth at regular intervals gives insight in growth kinetics, which can be a helpful tool for estimating coral aquaculture productivity (see next section). Nevertheless, our results are specific for the particular genet of *G. fascicularis* that was used for the experiments. Since the magnitude of the effect of a given factor on coral growth depends both on the species and on the genet, the results on a single genotype cannot be extrapolated to the species level and certainly not to the level of Scleractinia (**Chapter 6**). Therefore, our results should not be used as a blueprint for coral aquaculture. The value of our experiments for coral aquaculture lies in the fact that knowledge of mechanisms and relative importance of factors allows targeted optimization of coral growth. Hence, the information in this thesis can be used as a blueprint for targeted optimization studies. In particular, knowledge on interactions allows targeted optimization of coral growth and maximization of productivity for coral culture. An example of this in this thesis is the interaction between light and water flow, which implies that these factors should be evaluated simultaneously and not separately. The aquarium industry will thus also benefit from multi-factorial experiments, since such experiments will uncover interactions and facilitate extrapolation of the results to different coral culture conditions (within the framework of the experiment) and will thus increase the value of the experiment for coral culture purposes.

Practical experiments could additionally be improved by shortening experimental time, e.g. by using a fast-growing genet of each species to be cultured and/or high resolution measurements of coral growth. As a result of genetic variability, each coral species and

genet should be optimized separately (principles are the same, specific optimal combination of conditions is variable between species and genets). Depending on the extent to which different species or genotypes need different culture conditions for optimal growth, individual species and genotypes should be kept in separate culture systems. Drawbacks of such 'monoculture' is a higher vulnerability of the population to stressors due to the lack of genetic diversity. Therefore it can be recommended to keep a 'back up' of each species in a different system.

5.2 Growth models

Knowledge of growth kinetics of a coral species can be used as a tool to predict growth in mariculture. Theoretically, if a model appropriately describes growth there should be no correlation between the growth rate constant and the size of the coral. However, as shown in **Chapter 3**, specific exponential growth rate of *G. fascicularis* decreases with time and increasing colony size. With the help of more sophisticated modeling approaches, a more constant specific growth rate could be reached. Such a model could enable more realistic estimates of long-term coral production and ease the comparison of growth rates between culture conditions. Growth of *G. fascicularis* (expressed as the increase in buoyant mass) was found to have a better fit to a surface-dependent growth model (Sipkema et al. 2006) than to an exponential growth model (see Table 1). Since calcification only occurs in the calicoblastic epithelium of the coral tissue, skeletal growth is limited to the surface area of the coral's tissue. It is therefore the size of the surface and not of the volume of the colony that is determining the rate of calcification (Bak 1976). As result, the most appropriate model to describe the skeletal growth of a mound-shaped coral like *G. fascicularis* must be a model describing surface-area related growth of half a sphere (which is an approximation of the shape of a mound-shaped coral). Another approach is using surface area as a growth parameter for two-dimensional growth analysis. The circumference-dependent growth model (Sipkema et al. 2006) also had a higher fit than the exponential growth model (Table 2) for the corals in the 10 cm s^{-1}, 20 cm s^{-1} and 25 cm s^{-1} flow treatment. The selection of a growth model and its corresponding growth parameter depends on whether

7

one want a two-dimensional or three-dimensional prediction of growth. Different models will apply to different shapes of corals.

Table 1: *Accuracy of fit of the exponential growth model and surface-dependent growth model to the buoyant mass data (week 1 to week 42) of each experimental flow treatment. Buoyant mass is used as a proxy for volume. Adjusted R2 value were obtained using Sigmaplot 8.02.*

	flow treatments			
Growth models	**0 cm s^{-1}**	**10 cm s^{-1}**	**20 cm s^{-1}**	**25 cm s^{-1}**
based on buoyant mass				
Exponential growth	0.855	0.958	0.976	0.935
Surface-dependent growth	0.877	0.967	0.983	0.944

Table 2: *Accuracy of fit of the exponential growth model and surface-dependent growth model to the surface area data (week 1 to week 42) of each experimental flow treatment. Adjusted R2 value were obtained using Sigmaplot 8.02.*

	flow treatments			
Growth models	**0 cm s^{-1}**	**10 cm s^{-1}**	**20 cm s^{-1}**	**25 cm s^{-1}**
based on surface area				
Exponential growth	0.652	0.939	0.945	0.938
Circumference-dependent growth	0.553	0.949	0.949	0.946

Besides using an appropriate growth model, reliable protocols are needed for determination of growth parameters. In the case of *G. fascicularis*, actual surface area is not easily measured due to the protruding calyxes of the coral. Therefore, simplifications are made and surface area in our studies was defined as the projected surface area of the coral, i.e. that receives light perpendicular from above. Besides using buoyant mass, which requires an expensive under-weighing balance, coral biomass could also be approximated by using the volume displacement technique to measure coral volume. The volume of the coral is equal to the volume of water that is displaced after adding the coral to a known volume of water. Alternatively, the ecological volume (i.e. the water volume occupied between the branches or polyps of the coral species) could be determined (Rinkevich and Loya 1983) by measuring e.g. height, width and length of the coral and assuming a regu-

lar shape (Bongiorni et al. 2003b). (Ecological) volume is a useful parameter for aquarium purposes, since it gives a reliable estimate of the space it needs in the aquarium system that cannot be used by other organisms

5.3 Potential cost reductions for coral aquaculture

5.3.1 Adjusting environmental factors

Besides optimizing coral growth, also cost reductions can be made when knowing the interactions between factors. In such case, factors can be adjusted to each other to reach maximum benefit for coral production. Examples of this are:

a. **Irradiance and water flow,** as demonstrated in **Chapter 5**. A significant interaction was found between irradiance and water flow, confirming that the stimulating effect of light on coral growth of *G. fascicularis* colonies is much more enhanced at high(er) water flow rates. It seems even that coral growth is inhibited at the combination of high irradiance with low flow. Consequently, it is a waste of money and energy to apply a high irradiance without applying sufficient flow, since there will be no proportional return in coral production. However, one needs to remain cautious, because different coral species may respond differently. These results stress the importance of the availability of sufficient water flow for optimal light utilization for growth.
b. **Irradiance and aragonite saturation state**, as demonstrated by Marubini et al. (2001). A significant interaction was found between irradiance and aragonite saturation state, confirming that the stimulating effect of light on coral growth is much more enhanced at a high(er) aragonite saturation state. Coral growth of *Porites compressa* benefited from a higher aragonite saturation state at very high irradiances (700 μE m-2 s-1). Consequently, application of more light is more beneficial when having a higher aragonite saturation state. The costs of running a calcium reactor at a higher level and using more coral sand to fuel it are nothing compared to the benefits of a higher coral production. Most of the calcium carbonate that is dissolved inside the calcium reactor will eventually return in the form of newly formed carbonate skeleton.

c. **Light and feeding**. In the aquarium world, for many years the belief persisted that more light resulted in more growth and that additional feeding was not important. Several studies showed that coral growth can benefit from additional heterotrophic feeding (Houlbrèque et al. 2003, 2004, Ferrier-Pagès et al. 2003). Ferrier-Pagès et al. (2003) reported that Stylophora pistillata had increased growth with increasing irradiance (80 and 300 µE m^{-2} s^{-1}) both under fed and starved conditions. On the other hand, additional feeding could be a waste of money when it is not additive. For example, Lavorano et al. 2008 did not find a difference in growth of *P. damicornis* between different feeding regimes (0, 2000, 4000 and 8000 art l^{-1}, 5 days a week, 5 hours feeding time) after 126 days. This indicates that feeding was not additive for this species under the circumstances of the experiment. Possibly, the level of irradiance (200 µE m^{-2} s^{-1}) was limiting in this study, since in a later study they did find a difference in growth between feeding regimes at an irradiance of 300 µE m^{-2} s^{-1}. Corals fed with 2000 art l^{-1} grew significantly more than corals fed with 0 or 500 art l^{-1} (**Chapter 6**, Sylvia Lavorano pers. comm.). Similarly, the data of Houlbrèque et al. 2003 indicate that neither control nor fed corals of *S. pistillata* had increased growth with increasing irradiance (80, 200 and 300 µE m^{-2} s^{-1}). It is not known which factor was limiting coral growth in that study.

An outline of costs should be made for each situation and coral species to estimate the benefits for increased heterotrophic feeding for coral growth against the possible drawback of needing more filtration power to maintain proper water quality. A solution for maintaining proper water quality while maintaining high feeding levels is using a plankton friendly aquarium system that stimulates the natural build-up of plankton populations and enhances natural cycles for nutrient decomposition inside the aquarium.

5.3.2 Adjusting aquarium technology

Also cost reductions can be made by using efficient lighting i.e. a cost-efficient lamp type and cost-efficient lighting strategy (see section 6.4.2). Many different types of artificial light sources are available nowadays, i.e. with different light outputs, light spectra (often described by the manufacturer as color temperature in degrees Kelvin, which is a rough approximation for light spectrum) and energy-saving properties.

Blue and white light were found to promote more skeletal growth in *P. damicornis* and *Montipora verrucosa* than green or red light in (Kinzie et al. 1984). Whether blue light is the deciding factor in promoting growth remains to be investigated, since Riddle (2008) did not find a correlation between the amount of blue light and growth rate of *Acropora solitaryensis*. In contrast, consistent with the findings of Kinzie et al. (1984), Riddle (2008) found a negative correlation between the amount of red light and growth rate. Since red light does not penetrate deeply into the water column (i.e. it is absorbed at relatively shallow depths), many corals are found in environments deficient in red light. 'Strong' red light is therefore unnatural and might be inhibiting coral growth. A blue-white light spectrum is often used in coral aquaculture and is thought to be most efficient light spectrum for coral growth.

A new entrant into the market of aquarium lighting is LED (Light Emitting Diode) lighting. Besides having a lower energy consumption per amount of light emitted, LED lighting has many other advantages over traditional light sources (such as fluorescent lighting and metal halides), including a longer life time and the nearly complete absence of heat build-up. Besides this, LED lighting is also available in many different colors and light spectra, it is possible to adjust the light output by dimming and their light is focused into the aquarium without the use of external reflectors. The downside of LED lighting is the initial costs of purchase. However, due to the longer life time, this initial cost of purchase can be recovered in time with low running costs (electricity usage reduction) and less bulb replacements. Although there is yet little (scientific) experience with LED aquarium lighting, the technique looks promising and awaits investigation.

Appeal to market

Next to the efficiency of coral growth and its production costs, the economics of the culture of scleractinian corals for commercial purposes is also affected by the size, price, morphology (shape) and coloration of the final product (Delbeek 2001). Of these factors, color is by far the most important to increase the chance of market penetration and gaining the largest share of that market (Delbeek 2001). Pronounced coloration of corals (i.e. bright blue, green, purple or pink) is mostly due to the presence photo-protective pig-

ments (e.g. fluorescent pigments and chromo proteins) that are synthesized in response to stressful amounts of light, as occurs in nature in shallow reef environments (Salih et al. 2000). Thus, although “more light” might not be needed for more coral growth and might even stress corals, more light can produce nicer colors for the aquarium industry. However, also other factors, such as genetics, ultraviolet radiation, light spectrum and even low amounts of light can trigger the production of colorful pigments (Schlichter et al. 1986; Dunlap and Shick 1998; D’Angelo et al. 2008). The exact mechanisms for triggering the production of different coral pigments remain largely unknown. Future research on this topic would benefit the coral aquarium industry.

6 Main conclusions

Objective 1: Does water flow affect the skeletal growth of corals? Is this effect related to photosynthesis?

Yes, increasing water flow increases the skeletal growth of *Galaxea fascicularis*. The extent of the effect depends on the irradiance level. Especially at high irradiance, the effect of water flow is important to relieve oxidative stress. The mechanism of enhancement of coral growth with increasing water flow was not conclusively demonstrated to be related to photosynthesis at each irradiance level (90, 300 and 600 µE m^{-2} s^{-1}). It is suggested that it might be related to the relief of limitation by algal overgrowth, the supply of (in)organic nutrients, and, at high irradiance levels, the relief of oxidative stress.

Objective 2: Does irradiance affect the skeletal growth of corals? Is this effect related to photosynthesis?

Yes, skeletal growth of *G. fascicularis* increases with increasing irradiance. The enhancing effect of irradiance on skeletal growth is positively related to net photosynthesis. However, this relationship was not directly proportional, indicating that the enhancement of calcification by light is not entirely photosynthesis-driven. At high irradiance levels, there was a discrepancy between the potential energy produced in photosynthesis and energy used for skeletal growth, which could potentially be overcome by removing limitations for other factors such as heterotrophic feeding, aragonite saturation state and water flow.

Objective 3: Does light duration affect the skeletal growth of corals? Is this effect related to photosynthesis?

The effect of light duration on skeletal growth of *G. fascicularis* is not yet known under light-limiting circumstances. Light was probably in excess. Corals in the 24 hour light treatment were not able to adapt to prolonged light duration under light saturating conditions: they bleached immediately and died after 14 weeks. Corals in the 16 hour light treatment were able to adapt to prolonged light duration under light saturating conditions by decreasing their hourly rate of photosynthesis, possibly as a result of self-shading.

Since neither daily net photosynthesis nor skeletal growth were different between different photoperiods (8 hour versus 16 hour), it is suggested that enhancement of calcification is mediated by photosynthesis. Conversely, the corals in the 16 hour light treatment had access to more photosynthetic carbon (higher daily P/R ratio), which did not have a positive effect on the growth of these corals. It is suggested that the increased availability of photosynthetic carbon is allocated towards defense mechanisms against oxidative stress. However, this should be topic of future research.

Objective 4: Is there an interaction between the influence of irradiance and water flow on the skeletal growth of corals?

Yes, a significant interaction was found between the influence of irradiance and water flow on the skeletal growth of *G. fascicularis*, indicating that this effect is more than additive. Water flow is of increasing importance to coral growth with increasing irradiance levels. A coral grown at high irradiance but low flow might grow at a comparable rate as a coral grown at a lower irradiance but high flow. The enhancement of coral growth could not conclusively be related to net photosynthesis. It is suggested that enhancement of coral growth with increasing flow is related to the relief of oxidative stress and therefore reduced energy allocation towards photo-protective mechanisms.

Objective 5: Biology and economics of coral growth - implications for coral aquaculture

Knowledge of the factors controlling coral growth can contribute to developing cost-efficient coral aquaculture. As a result of the large amount of factors that can either stimulate, limit or inhibit coral growth, optimization of coral aquaculture implies close fine-tuning of different factors. Since many of these factors also interact, it is proposed that future research should focus on multi-factorial rather than single factor studies. Since each species and each genotype will require a different combination of values to maximize its growth rate, efficient high-density coral culture is best achieved by having the individual species and genotypes in separate culture systems. Growth models describing coral growth kinetics of a particular species can be used as a tool to design coral aquaculture systems and to calculate the costs of aquaculture until market size.

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Summary

Chapter 1: Introduction

Scleractinian or stony corals are sessile colonial modular animals that live in symbiosis with unicellular dinoflagellates, known as zooxanthellae. They can feed both phototrophically (i.e. by using translocated photosynthetic carbon from their zooxanthellae) and heterotrophically (i.e. by capturing zooplankton or taking up dissolved organic matter), allowing for tight nutrient recycling and enabling their survival in an oligotrophic environment. Scleractinian corals are key organisms of tropical coral reefs and responsible for building the large carbonate reef frame work that provides habitat to thousands of other reef organisms.

The intriguing reef ecosystem is increasingly threatened by both natural and anthropogenic factors. Concurrently with the gradual decline of coral reefs, there is a growing interest in keeping this delicate ecosystem in aquaria. The trade in marine ornamental species has become a multi million dollar industry. To reduce pressure on the reef, it is the policy of zoos and aquaria to display organisms that originate from sustainable breeding facilities. In support of the development of cost-effective aquaculture of corals for aquaria a project (CORALZOO, EU-funded, nr. 012547) was established to provide a scientific basis for coral husbandry techniques. The aim of this thesis was to study the influence of light and water flow on coral growth.

Chapter 2: Water flow

Being sedentary marine invertebrates, scleractinian corals are both dependent on and constrained by the presence of water flow. Water flow affects the exchange rate of dissolved gasses and (in)organic nutrients, prey capture efficiency and the removal of sediment and mucus. Growth rates of corals will therefore be determined by the sum of effects that flow exerts on coral physiology. Since scleractinian corals are symbiotic organisms, constricted to the euphotic zone of the coral reef and relying for a large part on photosynthetic energy from their symbionts, the effect of water flow was expected to be related to a water flow modulated effect on photosynthesis. Therefore, the effect of different flow regimes on long-term growth of *Galaxea fascicularis* was studied and related to respirometric measurements of photosynthesis and respiration. Four series

of ten coral nubbins of *Galaxea fascicularis* were exposed to four different flow regimes (0 cm s^{-1}, 10 cm s^{-1}, 20 cm s^{-1} and 25 cm s^{-1}) for 42 weeks at an irradiance of 90 µE m^{-2} s^{-1}. Coral growth was measured as buoyant mass, surface area and polyp number. Net photosynthesis and respiration were measured at their specific flow conditions inside a respirometric flow cell.

In the absence of flow, corals grew significantly slower and appeared unhealthy. In the presence of flow (10, 20 and 25 cm s^{-1}), growth rates significantly increased. However, no proportional increase of growth with increasing water flow was observed: average specific growth rate was not significantly different between 10 and 20 cm s^{-1}, while it was significantly higher at 25 cm s^{-1}. From 10 to 25 cm s^{-1}, average net photosynthetic rate decreased and average dark respiration rate increased. As a result, scope for growth based on phototrophic carbon decreased with flow.

Differences in growth rates could not be explained by a flow-modulated effect on either photosynthetic rate or photosynthetic carbon left for growth. It is suggested that higher flow rates reduce the chance of disturbance of coral growth by competing algae, allowing corals to grow more often with the maximum specific growth rate that is possible under the given environmental conditions. Also other effects of increased flow, such as increased respiratory rates and increased (in)organic nutrient uptake, might have been co-responsible for the increased growth of the corals in 25 cm s^{-1}. It is expected that phototrophic feeding and the modulation of photosynthesis by water flow will become more important at high irradiance.

Chapter 3: Irradiance

As a consequence of living in symbiosis with phototrophic algae, corals are dependent on light. According to the light-enhanced calcification theory, calcification of the coral host is enhanced by photosynthesis of the zooxanthellae. However, the exact mechanism of the enhancement of calcification by photosynthesis is still a matter of debate. Although several researchers found a positive correlation between skeletal growth and irradiance, it was not yet demonstrated whether the enhancing effect was mediated by photosynthesis throughout a range of light intensities. To provide experimental evidence for mediation of

light-enhanced calcification by photosynthesis, the relation between irradiance, skeletal growth and net photosynthesis was studied in *Galaxea fascicularis*. Coral nubbins of *Galaxea fascicularis* were exposed to four different light treatments that provided an irradiance of 38, 68, 166 and 410 μE m^{-2} s^{-1} for 294 days. Growth of these nubbins was measured as buoyant mass at different time intervals. A light-saturation curve for photosynthesis was measured in a respirometric flow cell using a *Galaxea fascicularis* colony grown at 60 μE m^{-2} s^{-1}.

Both skeletal growth and photosynthesis increased with irradiance. However, skeletal growth and photosynthesis were not directly proportional. The increase of specific growth rate with irradiance was less than expected based on the increase in net photosynthetic rate with irradiance. This discrepancy between potential energy produced in photosynthesis and energy used for skeletal growth indicates that skeletal growth is not limited by photosynthetic potential at high irradiance levels. It is suggested that either growth became inhibited by light or limited by other factors such as heterotrophic feeding or the availability of bicarbonate (i.e. aragonite saturation state).

Chapter 4: Photoperiod and photoacclimation

The quantity of light that is available for coral growth is not only determined by irradiance level, but also by the length of the photoperiod. Coral nubbins of *Galaxea fascicularis* were cultured for a period of 18 weeks at different photoperiods (8h 150 μE m^{-2} s^{-1}:16h dark, 12h 150 μE m^{-2} s^{-1}:12h dark, 16h 150 μE m^{-2} s^{-1}:8h dark, 24h 150 μE m^{-2} s^{-1}:0h dark) and irradiances (8h 150 μE m^{-2} s^{-1}:16h dark, 8h 225 μE m^{-2} s^{-1}:16h dark and 8h 300 μE m^{-2} s^{-1}:16 h dark). Growth during the experiment was determined by measuring buoyant mass. More light, either as irradiance or as photoperiod, did not result in more growth. Since in this experiment light was found not to be limiting for growth, the experiment was used to study photoacclimation of *Galaxea fascicularis* to different photoperiods under light-saturating conditions. To detect photoacclimation of the corals to an increased light duration, net photosynthesis, dark respiration, daily P/R ratio, zooxanthellae density and chlorophyll content were measured for corals grown at 8h 150 μE m^{-2} s^{-1}: 16h dark and corals grown at 16h 150 μE m^{-2} s^{-1}:8h dark.

Continuous lighting (24h 150 µE m^{-2} s^{-1}:0h dark) resulted in immediate bleaching and the corals died after 14 weeks. The corals in the other treatments were able to adapt to the prolonged light duration. The corals grown at 16h 150 µE m^{-2} s^{-1}:8h dark had decreased their hourly rate of photosynthesis compared to the corals grown at 8h 150 µE m^{-2} s^{-1}:16h dark. As a result, daily net photosynthesis was not significantly different between the 8 hour light and 16 hour light treatment, showing photoacclimation of the corals, which may explain the comparable growth rates. Photoacclimation was not achieved by changes in chlorophyll A or zooxanthellae density, since no significant differences were found in these parameters.

Based on the results of this study it is proposed that *Galaxea fascicularis* exhibited a form of self-shading, which is a known behavioral response of several coral species to e.g. excess light. Possibly, as a result of this photo-protective response, growth rates were not further reduced when exposed to an extended photoperiod under light saturating conditions.

Chapter 5: Interaction between water flow and irradiance

Based on the findings in Chapter 2, it was expected that the modulation of photosynthesis by water flow would become more important for coral growth at higher irradiance levels. To study the interaction between irradiance and water flow on coral growth and photosynthesis, a 2x2-factorial design was employed (Chapter 5): high irradiance (600 µE m^{-2} s^{-1}) vs. intermediate irradiance (300 µE m^{-2} s^{-1}) and high flow (15-25 cm s^{-1}) vs. low flow (5-10 cm s^{-1}). Growth was measured as buoyant mass and surface area. Photosynthetic rates of each coral was measured at its specific light and flow conditions inside a respirometric flow cell. A significant ($p<0.02$) interaction between light and water flow was found with respect to specific growth rate measured as buoyant mass, while the interaction between light and water flow on the specific growth rate of the surface area reached significance at a probability level of 9%. Water flow stimulated growth more at 600 µE m^{-2} s^{-1} than at 300 µE m^{-2} s^{-1}. The corals had the highest growth rate at high irradiance in combination with high flow. Net photosynthetic rates were not significantly different between treatments. Enhancement of coral growth with both increasing water

flow and increasing irradiance could not be explained by a corresponding increase in net photosynthetic rates. Possibly, the need for costly photo-protective mechanisms at low flow regimes can explain the differences in growth with flow.

Chapter 6: The biology and economics of coral growth

To provide more insight in the contradictory findings of the relationship between the amount of light and coral growth, an overview of factors that control coral growth was made. Besides numerous environmental factors that can potentially limit or inhibit coral growth, also genetic variability plays a role in determining coral growth rates. Since the magnitude of the effect of one factor often depends on the other, it is of importance for the future to perform multi-factorial experiments to provide more insight in interactions between factors. Due to the multiple interactions, optimization of coral aquaculture implies close fine-tuning of different factors. Each species and each genotype will require a different combination of values to maximize its growth rate. Growth models describing coral growth kinetics of a particular species can be used as a tool to design coral aquaculture systems. A proper growth model can reliably extrapolate the size of a coral nubbin to its market size and therefore help to calculate the costs of aquaculture and help determining the best culture strategy. It is shown that optimization can be rewarding, since for example optimizing feeding of Seriatopora caliendrum increased productivity with 35% while production costs increasing only with 15%.

Chapter 7: General discussion

The potential importance of interactions between factors for the understanding of coral growth and improvement of coral aquaculture is stressed. Moreover, a limitation concept for coral growth is introduced to explain different results for the relationship between light and skeletal growth. The following working mechanism for coral growth is proposed: the coral-algal symbiosis will adapt to the prevailing light regime by optimizing light capture and light processing at different levels of the organism, in an attempt to maximize light use. Depending on external environmental conditions, photoacclimation may not always be sufficient to obtain light saturation for coral growth. Under light-limited conditions, the

coral will try to compensate for reduced carbon/energy input by heterotrophic feeding or reducing their losses. Under light-saturating conditions, other environmental factors start to limit or inhibit coral growth, e.g. inorganic nutrients, heterotrophic food, inorganic carbon or the presence of water flow. Water flow is very important in both situations, since without flow it is difficult to adapt to either heterotrophic living (no supply of food) or phototrophic living (light stress). Finally, it is discussed how this thesis work can contribute to the improvement of aquaculture of corals. The information in this thesis should not be used as a blueprint for coral aquaculture, however, its value lies in providing a blueprint for targeted optimization studies of coral aquaculture.

Samenvatting

Hoofdstuk 1: Introductie

Steenkoralen zijn sessiele, kolonievormende en rifvormende dieren die in symbiose leven met eencellige algen, ook wel zoöxanthellen genoemd. Een koraalkolonie kan zich daardoor zowel fototroof (i.e. gebruik makend van de producten die de zoöxanthellen produceren tijdens fotosynthese) als heterotroof (i.e. gebruik makend van zelf gevangen zooplankton of opgenomen opgeloste organische stoffen) voeden. Door efficiënte recycling van nutriënten is het koraal in staat om te overleven in een nutriënt-arme omgeving.

Steenkoralen bouwen kalkskeletten door het afscheiden van calciumcarbonaat en zijn daardoor verantwoordelijk voor de bouw van de enorme kalkstructuren die aan duizenden andere riforganismen een habitat bieden. Dit bijzondere ecosysteem wordt in toenemende mate bedreigd door zowel natuurlijke als antropogene factoren zoals klimaatverandering, destructieve vistechnieken, overbevissing en vervuiling. Door de geleidelijke achteruitgang van de koraalriffen en het groeiende bewustzijn van de waarde van dit ecosysteem, is er een groeiende interesse ontstaan voor het houden van dit kwetsbare ecosysteem in aquaria. De handel in koralen en andere riforganismen (marine ornamental species) is lucratief geworden.

Ook bij publieke aquaria is er een trend om levende koraalriffen aan het publiek te laten zien. Om de druk op natuurlijke koraalpopulaties te verminderen, hebben dierentuinen en publieke aquaria het beleid om zoveel mogelijk dieren te laten zien die voortkomen uit duurzame kweek programma’s. Ter ondersteuning van de ontwikkeling van duurzame kweek programma’s voor steenkoralen voor aquaria, is er door een groep Europese dierentuinen en onderzoeksinstellingen een project (CORALZOO, EU-gefinancierd, nr. 012547) uitgevoerd om een wetenschappelijke basis voor koraalkweek technieken te verschaffen. Dit promotieonderzoek maakte deel uit van het CORALZOO onderzoeksprogramma. Het doel van dit promotieonderzoek was het bestuderen van de invloed van licht en stroming op koraalgroei. De Indopacifische soort *Galaxea fascicularis* werd hierbij gebruikt als model-organisme.

Hoofdstuk 2: Stroming

Doordat steenkoralen een sessiel bestaan leiden zijn ze voor veel processen afhankelijk van de aanwezigheid van stroming. Stroming beïnvloedt de opname en afgifte van opgeloste gassen en (an)organische nutriënten, de efficiëntie waarmee plankton gevangen wordt en de verwijdering van sediment en koraalslijm (mucus). De groeisnelheid van koralen kan daarom worden beschouwd als de resultante van de verschillende effecten die stroming heeft op de fysiologie van koralen. Aangezien symbiotische steenkoralen grotendeels afhankelijk zijn van de fotosynthetische capaciteit van hun symbionten, en daardoor beperkt zijn tot de eufotische zone van het koraalrif, werd verondersteld dat het effect van stroming op koraalgroei gerelateerd was aan fotosynthese.

Om deze reden werd in een eerste experiment de invloed van verschillende stromingsbehandelingen op de lange-termijn groei van *G. fascicularis* bestudeerd en gerelateerd aan respirometrische metingen van fotosynthese en respiratie. Vier reeksen van 10 nubbins (nubbins zijn fragmenten/stekken met een begingrootte van slechts één poliep) van werden blootgesteld aan vier verschillende stromingsbehandelingen (0 cm s^{-1}, 10 cm s^{-1}, 20 cm s^{-1} en 25 cm s^{-1}), gedurende 42 weken bij een lichtintensiteit van 90 µE m^{-2} s^{-1}. Koraalgroei werd gemeten als onderwatermassa, oppervlakte en het aantal poliepen. Netto fotosynthese en respiratie werden gemeten onder de specifieke stromingscondities van het koraal in een respirometrische stromingscel.

In afwezigheid van stroming groeiden de koralen significant minder en zagen ze er ongezond uit. In de aanwezigheid van stroming (10, 20 and 25 cm s^{-1}) waren groeisnelheden significant hoger. Echter, er werd geen consistente toename in groei met toenemende stromingssnelheid waargenomen: de gemiddelde specifieke groeisnelheid was niet significant verschillend tussen 10 cm s^{-1} en 20 cm s^{-1}, terwijl deze significant hoger was bij 25 cm s^{-1}. Van 10 tot 25 cm s^{-1} daalde de gemiddelde netto fotosynthese, terwijl de gemiddelde donker respiratie toenam. Als gevolg daarvan nam de groeipotentie (scope for growth) op basis van fototroof koolstof af met toenemende stroomsnelheid.

Verschillen in groeisnelheid konden niet worden verklaard door middel van een stimulerend effect van stroming op fotosynthese of de groeipotentie op basis van fototroof koolstof. Het is mogelijk dat hogere stroomsnelheden de kans op inhibitie van koraalgroei door

concurrerende algen verminderen, waardoor koralen vaker kunnen groeien met hun maximaal mogelijke specifieke groeisnelheid onder de heersende omstandigheden. Ook andere effecten van toenemende stroomsnelheid, zoals verhoogde respiratiesnelheden en verhoogde opname van (an)organische nutriënten, kunnen mede verantwoordelijk zijn geweest voor de toegenomen groei van de koralen bij 25 cm s^{-1}. Dit experiment is uitgevoerd bij een relatief lage lichtintensiteit. Vermoedelijk worden zowel de fototrofe levensstijl als het effect van stroming op fotosynthese belangrijker bij hogere lichtintensiteiten. Deze hypothese is getoetst in Hoofdstuk 5.

Hoofdstuk 3: Licht intensiteit

Door hun symbiose met fotosynthetische algen zijn koralen afhankelijk van licht. Volgens de theorie van lichtgestimuleerde kalkvorming (light-enhanced calcification) wordt de afscheiding van het kalkskelet door de koraal gastheer gestimuleerd door fotosynthese van de zoöxanthellen. Echter, het precieze mechanisme achter de stimulatie van kalkvorming door fotosynthese is nog steeds onderwerp van debat. Ondanks het feit dat verschillende onderzoekers een positieve relatie tussen skeletgroei en lichtintensiteit hebben gevonden, is het nog niet bekend of de stimulatie van groei over een reeks lichtintensiteiten gerelateerd is aan fotosynthese. Om experimenteel bewijs te leveren voor het belang van fotosynthese voor lichtgestimuleerde kalkvorming werd de relatie tussen licht intensiteit, skeletgroei en netto fotosynthese bestudeerd in *G. fascicularis*. Nubbins van *G. fascicularis* werden blootgesteld aan vier verschillende lichtbehandelingen die een lichtintensiteit Boden van 38, 68, 166 and 410 μE m^{-2} s^{-1} gedurende een periode van 294 dagen. Groei van deze nubbins werd gemeten als onderwatermassa over verschillende tijdsintervallen. Een verzadigingscurve voor licht en fotosynthese werd gemeten in een respirometrische stromingscel, gebruikmakend van een *G. fascicularis* kolonie die was opgegroeid bij 60 μE m^{-2} s^{-1}

Zowel skeletgroei als fotosynthese namen toe met lichtintensiteit. Echter, toename in skeletgroei en fotosynthese waren niet evenredig. De toename in specifieke groeisnelheid met lichtintensiteit was minder dan werd verwacht op basis van de toename van fotosynthese met lichtintensiteit. Dit contrast tussen de potentiële hoeveelheid energie

geproduceerd tijdens fotosynthese en de energie gebruikt voor skeletgroei wijst er op dat skeletgroei niet gelimiteerd wordt door fotosynthese bij hoge lichtintensiteiten. Mogelijke verklaringen voor de resultaten verkregen in deze studie zijn dat koraalgroei ofwel geïnhibeerd werd door licht, of werd gelimiteerd door andere factoren zoals de beschikbaarheid van heterotroof voedsel of the beschikbaarheid van bicarbonaat (de aragoniet verzadigingswaarde dat is niet hetzelfde!)

Hoofdstuk 4: Fotoperiode en fotoacclimatie

De hoeveelheid licht beschikbaar voor koraalgroei wordt niet alleen bepaald door de intensiteit van het licht, maar ook door de lengte van de fotoperiode. Het effect van fotoperiode op koraal groei is bestudeerd in Hoofdstuk 4. Nubbins van *G. fascicularis* werden gedurende 18 weken geweekt bij verschillende fotoperioden (8 uur 150 μE m^{-2} s^{-1}:16 uur donker, 12 uur 150 μE m^{-2} s^{-1}:12 uur donker, 16 uur 150 μE m^{-2} s^{-1}:8 uur donker, 24 uur 150 μE m^{-2} s^{-1}:0 uur donker) en verschillende lichtintensiteiten (8 uur 150 μE m^{-2} s^{-1}:16 uur donker, 8 uur 225 μE m^{-2} s^{-1}:16 uur donker and 8 uur 300 μE m^{-2} s^{-1}:16 uur donker). Groei gedurende het experiment werd gemeten als toename in onderwatermassa. Het aanbieden van meer licht, (zowel het verhogen van de lichtintensiteit als het verlengen van de fotoperiode), leidde niet tot meer groei in dit experiment. Omdat licht blijkbaar niet limiterend was voor groei, werd dit experiment gebruikt om de fotoacclimatie (adaptatie) van *G. fascicularis* aan verlengde fotoperiode onder licht verzadigende omstandigheden te bestuderen. Hiertoe werden de netto fotosynthese, donker respiratie, dagelijkse P/R ratio, zoöxanthellen dichtheid en chlorofyl concentratie bepaald van koralen die bij 8 uur 150 μE m^{-2} s^{-1}:16 uur donker groeiden en bij 16 uur 150 μE m^{-2} s^{-1}:8 uur donker.

Continue belichting (24 uur 150 μE m^{-2} s^{-1}:0 uur donker) resulteerde in onmiddellijke verbleking (bleaching) van de koralen en sterfte na 14 weken. De koralen in de andere lichtbehandelingen waren in staat zich te adapteren aan de verlengde fotoperiode. De koralen die groeiden bij 16 uur 150 μE m^{-2} s^{-1}:8 uur donker hadden een significant lagere fotosynthesesnelheid dan de koralen die groeiden bij 8 uur 150 μE m^{-2} s^{-1}:16 uur donker. Dientengevolge was de dagelijkse netto fotosynthese niet significant verschillend tussen de 8 uur en 16 uur licht behandeling, hetgeen wijst op adaptatie van de koralen aan

de verlendge lichtperiode (fotoacclimatie). Deze adaptatie verklaart mogelijk dat de groeisnelheden onder beide lichtperiodes vergelijkbaar waren. Fotoacclimatie werd niet bereikt door veranderingen in chlorofyl of zoöxanthellen dichtheid, aangezien hierin geen significante verschillen werden gevonden.

Onze gegevens duiden erop dat het mogelijk is dat *G. fascicularis* zichzelf beschermde tegen teveel licht door hun weefsel in te trekken en zichzelf hierdoor te 'beschaduwen', hetgeen een bekende gedragsmatige reactie is van verschillende koraalsoorten op bijvoorbeeld overmatig sterke belichting. Mogelijk is dit licht-beschermende mechanisme verantwoordelijk voor het feit dat de groeisnelheden niet verder reduceerden ten gevolge van verlengde fotoperiode onder lichtverzadigende condities

Hoofdstuk 5: Interactie tussen stroming en lichtintensiteit

Gebaseerd op onze bevindingen in Hoofdstuk 2, werd verwacht dat de modulatie van fotosynthese door stroming belangrijker zou worden voor koraalgroei bij hogere licht intensiteiten. Om de interactie tussen licht en stroming en het effect op groei en fotosynthese van *G. fascicularis* te bestuderen werd een 2x2 factoriëel ontwerp gebruikt: hoge lichtintensiteit (600 µE m^{-2} s^{-1}) versus middelmatige lichtintensiteit (300 µE m^{-2} s^{-1}) en hoge stroming (15-25 cm s^{-1}) versus lage stroming (5-10 cm s^{-1}). Groei werd gemeten als onderwatermassa en oppervlakte. Netto fotosynthese van koralen uit elke behandeling werd gemeten bij hun eigen specifieke licht en stromingscondities in een respirometrische stromingscel. Een significante interactie tussen licht en stroming werd gevonden voor specifieke groeisnelheid als onderwatermassa, terwijl de interactie tussen licht en stroming voor specifieke groeisnelheid als oppervlakte significantie bereikte bij een waarschijnlijkheid van 9%. Stroming stimuleerde groei meer bij 600 µE m^{-2} s^{-1} dan bij 300 µE m^{-2} s^{-1}. De koralen hadden de hoogste groeisnelheid bij hoge lichtintensiteit en hoge stroming. Netto fotosynthesesnelheden waren niet significant verschillend tussen de behandelingen. Toename van koraalgroei met zowel toenemende stroming als lichtintensiteit kon daarom niet worden verklaard met netto fotosynthese. Mogelijk kan de noodzaak voor kostbare licht-beschermende mechanismen bij lage stroming en hoge lichtintensiteit de verschillen in groei met stroming verklaren.

Hoofdstuk 6: De biologie en economie van koraalgroei

Om meer inzicht te verschaffen in de tegenstrijdige bevindingen betreffende de relatie tussen de hoeveelheid licht en koraalgroei is een overzicht van factoren die koraalgroei beïnvloeden gemaakt. Naast talrijke omgevingsfactoren die mogelijk koraalgroei kunnen limiteren of inhiberen, speelt ook genetische achtergrond een rol in het bepalen van de uiteindelijke groeisnelheid van koralen. Omdat de mate van het effect van één factor vaak afhangt van de andere factor, is het van belang voor de toekomst om te focussen op multi-factoriële experimenten om meer inzicht krijgen in de interacties tussen factoren. Optimalisatie van koraalkweek komt daarom neer op het nauw afstemmen van verschillende factoren. Iedere koraalsoort en ieder genotype vereist een specifieke combinatie van factoren met verschillende waarden om zijn groeisnelheid te kunnen maximaliseren. Groeimodellen die de groeikinetiek van een bepaalde koraalsoort beschrijven kunnen gebruikt worden als een instrument om koraal aquacultuur systemen te ontwerpen. Een nauwkeurig passend groeimodel kan betrouwbaar de grootte van een koraal nubbin extrapoleren naar de uiteindelijke marktgrootte en is daardoor bruikbaar voor het berekenen van de kosten van aquacultuur en het bepalen van de beste kweekstrategie. In dit hoofdstuk wordt aangetoond dat optimalisatie van factoren belonend kan zijn, aangezien bijvoorbeeld in het geval van optimalisatie van voer regime voor Seriatopora caliendrum de productiviteit verhoogd werd met 35%, terwijl productiekosten slechts stegen met 15%.

Hoofdstuk 7: Discussie

In de discussie wordt het potentiële belang van interacties tussen factoren voor inzicht in koraal groei en de verbetering van koraalkweek verder benadrukt. Daarnaast wordt een limitatie concept voor koraalgroei geïntroduceerd om de verschillende resultaten aangaande de relatie tussen licht en skeletgroei te verklaren. Het volgende werkingsmechanisme voor koraalgroei wordt voorgesteld: de koraal-algen symbiose zal adapteren aan het heersende lichtregime door middel van het optimaliseren van zowel de hoeveelheid licht die ingevangen wordt als de efficientie van de verwerking van dit licht, in een poging om het lichtgebruik te maximaliseren. Afhankelijk van externe

omgevingsfactoren, zal fotoacclimatie bij afnemende lichtintensiteit niet altijd voldoende zijn om lichtverzadiging voor koraalgroei te bewerkstelligen. Onder lichtgelimiteerde omstandigheden zal het koraal proberen zijn energie en koolstof tekort te compenseren door zich meer heterotroof te gaan voeden of verliezen te minimaliseren. Onder lichtverzadigende omstandigheden zullen andere omgevingsfactoren koraalgroei gaan limiteren of inhiberen, zoals bv anorganische nutriënten, heterotrofe voeding, anorganisch koolstof of de aanwezigheid van stroming. De aanwezigheid en de sterkte van de stroming is zeer belangrijk in beide situaties, aangezien het in de afwezigheid van stroming bijna onmogelijk is om te adapteren aan een heterotrofe levensstijl (want zonder stroming geen aanvoer van voedsel) of aan een fototrofe levensstijl (zonder stroming meer lichtstress). Tenslotte wordt bediscussieerd hoe dit proefschrift kan bijdragen aan de verbetering van de aquacultuur van koralen. De informatie in dit proefschrift biedt nog geen recept voor de aquacultuur van steenkoralen. De waarde van dit proefschrift ligt juist in het feit dat het een recept biedt voor de optimalisatie van aquacultuur van steenkoralen.

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Ronald Osinga, my daily supervisor and co-promotor – Without you this adventure could have never started! Starting up a new research line and building up a coral lab from scratch was not always easy. Thank you for your faith and optimism and for guiding me throughout this scientific adventure! I loved our discussions where we often strayed off from one interesting topic to another! Als het denkwerk maar goed is! I hope we get the chance to work together again in the future! I really like your research line as it developed during the last four years!
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Rene Wijffels, my second promotor – Being located at a different research group and in a different building, I unfortunately saw you mostly during our meetings. Thanks for responding so quickly to my papers while giving it a critical review. And thanks for giving me the opportunity to join the PhD trip to Japan!
Max Janse – Although you are not officially one of my supervisors, you are to me just as important as the rest! I learned a lot from you, both personally and professionally! And despite your busy schedule, being the curator of both the Ocean, the Bush ánd the Desert, you always had some time left for discussions. It was great to be working with you and I hope we can work together more in the future!

Starting to work at Aquaculture and Fisheries (AFI) meant entering a men's world with a high cultural diversity. I have had colleagues from Portugal, Eritrea, Tanzania, Bangladesh, Colombia, Iran, Germany, Spain, Vietnam, Indonesia, India, Greece and Poland. And, being one of the few females in our group, I have been asked many times to be one of the paranimphs at the graduation ceremony of our male PhD students. This gave me the opportunity to practice being on stage in the Aula in front of many people for not less than 5 times!! Thanks for this Titu, Yonas, Iyob, Mohammad and Pascal! I would also like to thank all the other people from AFI for my pleasant time at AFI. In particular, I would like to thank Ronald Booms for his support in the lab and for his critical thinking when devising a new lab method for analyzing corals; Helene Willems for her positive presence and sociability; Pascal for being – for a long time – my only Dutch PhD colleague and of course for helping me with SAS (statistics), Bob for his practical insights and helping me devise an efficient coral tissue remover setup, and Julia and Christos for being dear colleagues and also very nice friends!

Although our Coral Research group started out from scratch, it soon became quite popular among BSc and MSc students and our coral lab space also grew significantly. First located next to the catfish in the big hall of the Hatchery, later on we got assigned our own coral room. Since keeping salt-water aquaria was a time-consuming job next to the regular scientific work, we hired Tim Wijgerde (who is also a coral reef aquarium hobbyist) to take the role of aquarist and to assist in research projects. He helped to improve our aquarium systems even further and developed our aquarium systems into prosperous reef aquaria. When Tim left, his job was taken over by our former MSc student Anneke and - after she left - by the people from the Hatchery (Sietze Leenstra, Wian Nusselder, Aart Hutten and Sander Visser). I would like to thank Tim, Anneke and the Hatchery people for their supportive work and the Hatchery people also for being so hospitable to host our corals amidst all the fish in the Fish Palace! Thanks also go to Nico Post who, as a aquarium hobbyist, donated many colonies of *Seriatopora caliendrum* to our project. This species proved to be a much quicker grower and also an easier species to work with compared to *Galaxea fascicularis*. Future research in our lab will certainly benefit from using this species!

I would also like to thank my students that helped me during my PhD work: Bas, John, Anneke, Rosa, Nanne and Ben. Thanks for all your input and efforts! You all did a large amount of work and without you it would not have been possible to finish a PhD thesis on coral growth in time!!! I hope we will stay in touch!
At the Technical Workplace of Zodiac I would like to thank Eric Karruppannan and Evert Janssen for their skilful help in (technically) developing research equipment tailored to my research needs. It was great to come to you and develop my research needs into a concrete plan and design! For example, Eric and I designed and developed the first two prototypes of the respirometric flow cells that I used for my research, while Evert helped me with further improvements and contracting out the third one. I learnt a lot about technical stuff and really respect and appreciate your expertise! Next to that, I enjoyed drinking coffee with you, which is not only because you made much better coffee than that brownish liquid that comes from the regular University coffee machines! Special thanks go to Eric, for having a lot of fun, laughing a lot and all his support and encouraging words whenever I needed them! At the Technical Workplace of the Biotechnion I would like to thank Hans Meijer and Reinoud Hummelen for their electric support and providing equipment for powering the paddlewheel in my respirometric flowcell.

At Burgers Zoo, I would like to thank the whole animal keeper team of Burgers Ocean (Miranda, Arjan, Dennis, Leon, Marieke, Frank, Tim, Rob and Rik). Thank you all for facilitating my research and making me feel at home in Burgers Ocean! I appreciate everything you did, from feeding my corals to helping my students feel at home and find their way when I was not there! Thanks also to Arjan and Dennis for having a great diving holiday in Spain!! My sincere apologies for being so difficult to wake up in the middle of the night when you are ready to leave to Spain! ;)

Also, I would like to thank CORALZOO and my CORALZOO colleagues for giving me the opportunity to visit so many different public aquaria around Europe! Working on this project really changed my perception of (public) aquaria. I now know about all the effort that goes into designing and managing an attractive (coral) exhibit and can distill different

views on aquarium keeping. Thank you for your collaboration and your reflection on my research during my presentation at the CORALZOO meetings. I would also like to thank Muki Sphigel for hosting me at the National Centre for Mariculture in Eilat and Michal Raz-bahat and Buki Rinkevich for hosting me at the Israel Oceanographic and Limnological Research (IOLR) institute in Haifa during my trip to Israel.

Next to doing a PhD there is of course more in life… ! Although many of my non-scientific and/or non-Wageningen friends mabye did not understand what it was like to do a PhD, I would like to thank them for having a good time with me without talking about PhD-related stuff! However, it is funny to see that even a hairdresser in Wageningen knows what a PhD is… which to my opinion indicates how interwoven our scientific community is with the regular society in Wageningen.
I would like to thank Rogier, Marianne, Annemarie, Erna, Jan-Hans, Katja, Amancio, Cyrina and others for being a great party crew at the many parties and summer festivals that we went! Thanks Rogier for your helpful insights into management-related topics; such insights are helpful in every part of life and in any job, be it in science or in business! Thanks also Maria for having a great time together! I still miss you as my housemate!! And thanks Deby making me laugh and have silly talks! I also would like to thank my Wageningen and/or scientific friends for being able to share concerns and insights about doing a PhD and for looking at things the scientific way. Thanks Julia, Christos, Annette, Michael, David, Mara, Dirk (my coral collegue!), Rixt, Pedro, Jazzper, Michel, Heike, Joost, Anke, Aafke, Nigel, Robert, Bob, Irene, Sander, Rob, Haike, Marcel and everyone I have forgotten now in the rush of this moment.

Erwin, I met you only during the last few months of my PhD.. and I wish we had met much earlier!! Thanks for all you positive energy and for sharing so many morals and interests! I feel really at home with you! I hope we can and will enjoy each other for much much longer!!! Dikke kus!!!

Last but not least, I would like to thank my mum, dad and sister Kirsten for their confidence, support and pride during my scientific adventure. Additionally I would like to thank my grandparents Oma Kooi, Opa Kooi (R.I.P.) and Opa Schutter (R.I.P.) for their unconditional love and support. Also Tante Gert and Oom Aait get a special place here at the end of this chapter, since they are also as much as family to me!

As with most adventures, this scientific adventure made me grow both as a person and as a scientist. Now it’s time for a new adventure!

About the author

I was born on the 17th of March 1979 in Rhenen, The Netherlands. Already at a very young age my passion for marine biology became evident. During our summer holidays at the coast of Brittany (France) I spent endless hours playing in the rocky pools near the sea investigating sea life. Also many hours were spent writing adventure stories and fairy tales, resulting in my very first "book" at the age of 9. After finishing primary school in Elst, I went to the Christelijk Lyceum in Veenendaal where I received my Gymnasium B diploma in 1997.

From 1997 to 2004 I studied Biology at Wageningen University. I chose for Wageningen, because Wageningen University was known for its numerous international contacts and its nature-oriented people. While studying biology, I discovered that my curiosity for nature and investigative behavior was not at all uncommon! My study curriculum was very broad - ranging from entomology to developmental biology and immunology - but focusing on (experimental) zoology and physiology. My main interest was investigating the connection between form and function in curious animals. My study was put on a temporarily hold after I got confronted with my personal ethic during the Animal Handling course, which was compulsory for Animal Biologists. I spent one year working as educational assistant for the Human and Animal Physiology group and for the Cell Biology and Immunology Group. Motivated by my passion for seahorses and their curious reproductive behavior (the male seahorse gets "pregnant"), I resumed my studies and wrote my first research proposal on the developmental biology of juvenile seahorses to be executed in Rotterdam Zoo. Due to unforeseen circumstances, however, this project could not be continued. I had to switch subject and worked during my first thesis on the development of vascular system of zebra fish embryo's (*Danio rerio*) in response to high and low oxygen tension under supervision of Dr. Sander Kranenbarg at the Experimental Zoology Group. Using In Situ Hybridization we tried to identify the time of onset and localization of the expression of a certain gene involved in vasculogenesis (VEGF) in response to low oxygen tension.

Still motivated to pursue my studies on seahorse biology, I got in contact with Dr. Mark Lokman of the Marine Science Group at the University of Otago, New Zealand through Prof. Dr. Johan Verreth of the Aquaculture and Fisheries Group of Wageningen University. Dr. Mark Lokman was planning to study the reproductive biology of seahorses and was willing to host me for my second MSc thesis. Seahorses have an interesting reproductive biology, since the males get pregnant and keep the embryo's in their brood pouch (an external skin fold of their abdomen) in the until after hatching Since it was already known that the hormone prolactin plays a role in paternal care in sticklebacks, which species is closely related to the seahorse, this hormone was also implicated to play a role in paternal care of seahorses. In a preliminary research, I studied the influence of this paternity hormone on physiological, morphological and behavioral changes associated with male pregnancy, by employing a diverse array of techniques such as histology, immunocytochemistry and radio immunoassays.

After my graduation in March 2004, I spent six months in Curaçao, the Dutch Antilles, as the project coordinator of a coral reef conservation project. As part of this project, I developed and implemented an environmental education program for local schoolchildren and (co)-organized snorkel courses, swimming lessons, community meetings and reef monitoring events, in collaboration with the local government, NGOs, beach resorts and media. From my experience on Curacao I learnt that I love to work with different cultures, to live in an tropical environment and to make scientific knowledge accessible in creative ways.

After returning to the Netherlands, Prof. Dr. Johan Verreth offered me a PhD position within the European CORALZOO project at the Aquaculture and Fisheries Group at Wageningen University. This PhD project focused on studying the influence of abiotic

factors on the growth and physiology of stony corals in closed aquarium systems and most of the results of this project are presented in my second “book”, this thesis. Since there was no previous experience with corals or coral husbandry at Wageningen University, we started out with next to nothing in terms of facilities, hands-on experience and scientific expertise. Most of my long-term growth experiments were executed at the large coral aquaculture facilities in Burgers Zoo. Meanwhile, we also set up a coral aquaculture facility and research lab at Wageningen University. Additionally, in cooperation with the technical workplace of the Animal Science Group, I designed respirometric flow cells for physiological measurement of coral colonies under different light, flow and/or feeding conditions. Until now we attracted many students that want to do their MSc thesis with us!

After working hours, most of my leisure time is spent listening to music, meeting with friends, dancing at parties, cooking, and enjoying nature. Besides, I love to travel and do water sports (surfing, diving).

WIAS Graduate School

Training and Supervision Program

Education and Training 2005-2009 | ECTS

The Basic Package | **3**

- WIAS Introduction Course
- Course on philosophy of science and ethics

Scientific Exposure: conferences, seminars and workshops| among others: | **23**

Conferences

- International Society for Reef Studies, European Meeting, Bremen, Germany, 2006 (poster presentation);
- WIAS science day 2006, 2007 (poster and oral presentation);
- Dutch Coral Research Symposium at University of Amsterdam, The Netherlands, 2007 (oral presentation);
- Coral Husbandry Symposium at Burgers Zoo, The Netherlands, 2007 (poster presentation);
- CORE Mini-symposium, Munchen, 2008 (oral presentation, invited speaker);
- 11th International Coral Reef Symposium, Florida, 2008 (poster presentation;
- Second Symposium on The Ocean in a High-CO_2 World. Musée Océanographique, Monaco, 2008.

Seminars and Workshops

- 1st SECORE Workshop, Rotterdam, 2005;
- CORALZOO meetings: Genua 2006, Ancona 2007, Eilat 2008, Wageningen 2008 and Genua 2009 (oral presentations);
- EUAC congress, Lisboa, Portugal, 2005;
- Workshop Electrochemical measurements at Hach Lange, 2006.

	ECTS
In-Depth Studies: Disciplinary and interdisciplinary courses	**10**

- Aqualabs - Design and operation of Recirculation Technologies - Wageningen 2005;
- WIAS course: Biology underpinning animal sciences: broaden your horizon;
- WIAS Advanced Statistics course: Experimental Design;
- Coral Reef Ecosystems, University of Amsterdam.

Professional Skills Support Courses **3**

- Time Planning and Project Management;
- Organising and supervising MSc thesis work;
- Techniques for Writing and Presenting a Scientific Paper.

Research Skills Training **8**

- Preparing own PhD research proposal (2005);
- External training period at IOLR, Israel (2006);
- PhD study tour to Japan, visiting institutes and universities (2008).

Didactic Skills Training **15**

- Supervised Aquaculture and Fisheries practical (2 afternoons per year);
- Supervised 6 MSc students;
- Supervised excursion of student group to Burgers Ocean.

Total (1 ECTS credit equals 28h study load) **62**

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