The importance of feeding in determining the sensitivity of benthic invertebrates to future climate change in the tunicate species *Ciona intestinalis*

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2016

Thesis submitted in partial fulfilment of the Master's Degree of Science in Marine Biology – Marine Biodiversity

Front page: *In situ* picture of *Ciona intestinalis*. Picture from Institute of Marine Research.

Acknowledgements

This thesis was carried out in cooperation with the Institute of Marine Research (IMR), Benthic resources and process.

First of all I would like to thank my supervisor Raymond Bannister for giving me the opportunity to cooperate with IMR and for being a great motivator and very supportive in finding me a new project when my initial project proved not to work out. I also have to thank my main supervisor, Samuel Rastrick, for the opportunity to be a part of this interesting project. His knowledge and support has been much appreciated, Samuel has been a good mentor and of great help throughout this project. I also want to thank my co-supervisor, Tore Strohmeier, for his support and some very helpful advice and feedback on my thesis. I also would like to thank my co-supervisor, Svein Rune Erga, for being a great mentor and of tremendous support throughout this project. He has been very helpful in writing my thesis, giving me some very valuable advice and feedback.

There are several other people who have been of great support and help throughout this project that I also would like to thank. Thanks to Cathinka Krogness for all the help during the execution of this project. I also want to give a big thanks to my family and closest friends for all your support and positive reinforcement.

This thesis would not have been possible without the help from all of you.

Abstract

Anthropogenically induced global climate change is one of the worlds biggest threats today, with marine ecosystems particularly vulnerable. In the future, the ocean temperature is expected to rise with 2 °C followed by a reduction in pH by 0.3-0.4 units. The future function, survival and distribution of marine species are dependent on their physiological response to these future changes. This study aims therefore to investigate the importance of feeding in determining the sensitivity of the tunicate, *Ciona intestinalis*, to future climate change. This was done by looking into how ocean warming (OW) and ocean acidification (OA) would affect eco-physiological behaviour and the scope for growth in *C. intestinalis*. Our study was carried out by feeding the animals *ad libitum* using the natural system of surface water, with adequate content of seston, and deep water, with limited content of seston. The study comprised two experiments, where Exp. 1 kept the animals in four temperature treatments (7, 12, 15 and 17 °C) and Exp. 2 kept the animals in a combination of four temperature and pH treatments (12 °C with pH 8, 12 °C with pH 7.7, 15 °C with pH 8, and 15 °C with pH 7.7). Our results suggests that the combination of multiple stressors like OA and OW will impose a bigger threat when occurring together. *C. intestinalis* had a lower toleration level to the combined effects of OW and OA. Interestingly, the food limited animals scope for growth implied that they should be showing a positive growth response. However they were actually shrinking in size. When being subjected to stress from OW and OA the maintenance of homeostasis become more energetically expensive. *C. intestinalis* seemed to compensate for this energy demand by increasing their feeding and re-allocating energy away from processes like growth and respiration in order to survive. It seems evident that the availability of food is a considerable stressor affecting *C. intestinalis*, which can have various effects when occurring in combination with other stressors.

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1.0 Introduction

Marine ecosystems are known to be affected by human activities among which global climate change are one of the greatest threats (Glover & Smith, 2003; Halpern et al., 2007). Effects of climate change can cause major impact on not only individuals, but also the overall size, structure and functioning of populations and ecosystems (Portner & Knust, 2007). Some of these effects, like ocean warming (OW) and ocean acidification (OA), are more renowned and well accepted. The Intergovernmental Panel on Climate Change (IPCC) says with certainty that the ocean has increased its temperature since the late 19th century and that the Earths' surface has become successively warmer over the past decades. Since pre-industrial times global atmospheric concentrations of carbon dioxide (CO_2) has increased by 40 % and are predicted to rise even more by the end of the century. CO_2 has an insulating effect in the atmosphere and it is because of this that global sea-surface temperatures are predicted to rise even more by the end of this century. Seen on a global scale OW is largest in surface waters, and in the period 1971-2010 the temperature increased by 0.11 °C per decade in the upper 75 m. It is predicted that the global ocean will continue to warm during this century with about 0.6-2.0 °C in the upper 100 m and about 0.3-0.6 °C at a depth of about 1000 m. Furthermore, about 30 % of the anthropogenically derived CO_2 has been absorbed by the oceans which has altered the carbonate system and hence caused OA (IPCC, 2013). By the end of this century ocean pH is predicted to decrease from around 8.1 to 7.8-7.7 (Caldeira & Wickett, 2003).

The physiological responses of marine species to these environmental changes will affect their future function, survival and distribution. Their response are therefore of particular interest as it helps to define different scenarios where cellular homeostasis are maintained in response to predicted changes in temperature and pH (Calosi, Rastrick, et al., 2013; Doney et al., 2012; Fields et al., 1993; Harley et al., 2006; Portner & Farrell, 2008; Rastrick et al., 2014; Stillman, 2003; Sunday et al., 2011; Widdicombe & Spicer, 2008). Animals are adapted to survive within a temperature range called their 'thermal window'. When temperature shifts away from the animals' optimum it will result in reduced performance and eventually it can lead to death if the animals are unable to adapt to the new conditions.

If the change in temperature occurs slow enough, the animals might be able to adapt to the new conditions, or alternatively migration may occur. (Portner & Farrell, 2008).

With the increasing global warming and climate change, consequences are supposed to be seen both at individual levels as well as on ecosystem levels. The survival, distribution and functioning of benthic invertebrates in the future are dependent on their ability to maintain physiological homeostasis. However, it has been demonstrated by several studies that it is more energetically expensive to maintain homeostasis under OA and OW conditions (Calow, 1983, 1989, 1991; Calow & Forbes, 1998; Sokolova et al., 2012). Because of the increased cost of maintaining homeostasis, energy is diverted away from other major processes like activity, respiration, growth and reproduction (Li & Gao, 2012; Widdicombe & Spicer, 2008). These energetic trade-offs are well studied, however, there is a lack of knowledge on how these trade-offs are influenced by changes in the total amount of energy available to the animals through feeding. It has been suggested that the animal may compensate for the extra cost of maintenance through feeding, and would thereby not have to divert energy away from processes like behavioural activity, respiration, growth and reproduction (Mayor et al., 2015). Hence will the effects of OW and OA also be less. However, if the access to food is limited, like in deep-water or in Norway's oligo- to mesotrophic fjords, this could reduce the animals energy availability which might aggravate the effects of OW and OA (Bundy et al., 2009; Mayor et al., 2015).

Coastal marine environments are of great global importance, and are therefore a major concern due to the potential effects of anthropogenically induced climate change (Harley et al., 2006). As early as the 1990s it was predicted that rising temperatures associated with climate change may lead to a shift in the distribution and abundance of species coupled to their thermal tolerance, acclamatory capacity and adaptability (Fields et al., 1993; Lubchenco et al., 1993). Many later studies supports these predictions, however, the change in temperature are now seen as only one of several interacting biotic and abiotic drivers that are predicted to cause ecological changes in marine environments (Harley et al., 2006). With these alterations following climate change, many species are prone to undergo a shift in their ambient conditions. Additionally, organisms will continue to be exposed to other abiotic and biotic stressors, that can be both related and unrelated to global climate change (Byrne & Przeslawski, 2013). This will result in organisms being simultaneously exposed to

several different stressors, which could make future predictions hard to determine (Andrady et al., 2012; Przeslawski et al., 2005). However, the two most immediate stressors are temperature and acidification (Byrne & Przeslawski, 2013; Doney et al., 2012). Shifts in geographical ranges have already been reported in several different species moving towards higher latitudes in order to stay within their preferred temperature ranges. Marine environments might also be affected by these changes faster than terrestrial, given that range shifts occur faster in marine environments (Sorte et al., 2010; Walther et al., 2002). *Mytilus edulis* is an example of such a species, which over the past 50 years shifted its geographical range edge roughly 350 km to the north (Jones et al., 2010) and has now reappeared in Svalbard after a 1000 year absence (Berge et al., 2005). The blue mussel belonging to the east coast of USA has over the years experienced mass mortalities due to high temperatures during summer, which is thought to be the reason to this poleward shift (Jones et al., 2010). Both temperature and acidification have also been documented to affect early history life stages of several organisms. Early life history stages are particularly sensitive to environmental stressors, and are hence of great concern. It has been found that calcifying larvae are sensitive to both OW and OA, while non-calcifying larvae are more sensitive to OW (Byrne & Przeslawski, 2013). Changes in the thermal tolerance of an ectotherm species can also help explain the distribution along thermal gradients occurring with latitude, and hence help to predict the susceptibility of the species to environmental changes like elevated temperature and pH (Rastrick & Whiteley, 2011; Somero, 2002).

Elevated temperatures and seawater pCO_2 (carbon dioxide partial pressure) can affect the feeding behaviour, development and metabolism of marine benthic species (Widdicombe & Spicer, 2008). When atmospheric pCO_2 rise, the CO_2 that are dissolved in seawater will increase and hence lower the pH. The effects of acidification will in turn disturb the acid-base balance (Portner et al., 2004). However, most animals are to some degree able to maintain stability in the intracellular acid-base balance by using several ionic pumps and enzymes. These regulations require additional energy which can cause the animal to alter its development, feeding behaviour and/or metabolism to accommodate this energy demand (Garilli et al., 2015; Li & Gao, 2012; Portner et al., 2004; Whiteley, 2011; Widdicombe & Spicer, 2008). In a recent study (Li & Gao, 2012), the marine copepod *Centropages tenuiremis* was exposed to 1000 ppm CO_2, which caused its feeding rates to increase

compared to the controls. It seemed to compensate for the increased respiration rates resulting from the CO_2-exposure. External stressors, like OA, can reduce the energy available for processes like growth, and hence affect long-term reproductive output (Mayor et al., 2015). Marine secondary producers seem to increase their respiration and feeding rate as a response to OA in order to compensate for the extra energy cost resulting from increased acidity and CO_2 concentrations (Li & Gao, 2012). The effects of OW could also influence the energetic trade-offs within marine animals. A recent study (Mackenzie et al., 2014) concludes that OW will become a greater future problem for the bivalve *Mytilus edulis* than OA when food availability is limited. It was found that warming had an indirect effect on the shell strength of *M. edulis*, with energy being re-allocated away from shell formation to the increasing maintenance costs initiated by elevated temperature. These effects would also become amplified when food is limited and the mussels are already depending on their internal energy reserves (Mackenzie et al., 2014).

Currently, most studies focusing on the effects of global warming have either dealt with the globally elevated atmospheric pCO_2 or rising temperatures separately (Melzner et al., 2013; Small et al., 2010). There are few studies that have looked into how the combination of multiple climate driven stressors can affect the physiological response of marine invertebrates (e.g. (Calosi, Turner, et al., 2013; Mayor et al., 2015; Melatunan et al., 2011). Marine intertidal species may also have some resilience to the future changes in ocean chemistry since they are already adapted to periods of emersion, and thus to natural variations in temperature and pH (Byrne & Przeslawski, 2013; Melzner et al., 2009). This might suggest that they are already living near the limits of physiological tolerance and with further alterations in ocean chemistry they might exceed these limits (Deschaseaux et al., 2010). However, there are lacking research on how such species would deal with a permanent exposure to elevated temperature and pCO_2 (Calosi, Turner, et al., 2013; Widdicombe & Spicer, 2008). Information is also sparse on such species that have the potential to be more tolerant to future environmental changes. This would make them a good reference against more vulnerable species, which could give interesting information on physiological traits essential for future ecological success (Melzner et al., 2009).

Within this larger context, the aim of our study is therefore to investigate the importance of feeding in determining the sensitivity of the tunicate, *Ciona intestinalis,* to future climate

change. Specifically to investigate how OW and OA affect eco-physiological behaviour and the scope for growth in *C. intestinalis*. This will be done by feeding the animals *ad libitum* (meaning that the animals will be feeding freely on a self-regulatory level as in a natural system) using the natural system of surface water, with adequate content of seston, and deep water, with limited content of seston. By keeping the animals in different temperatures and a combination of different temperatures and pH treatments over four weeks, this study investigated the physiological response of *C. intestinalis* (metabolic rate, growth, clearance rate, energetics and scope for growth).

The ascidian *Ciona intestinalis* is a benthic suspension feeding animal living in dense aggregations attached submerged, solid substrate (both natural and artificial) in the sub-tidal zone. Ascidians have been used as invertebrate model organisms for over a century because of their close similarity to vertebrates (Passamaneck & Di Gregorio, 2005). There are huge variations in form among ascidians, differing from small colonial species to larger solitary species. There is still little knowledge on their feeding biology, which is part of the reason why most studies have focused on the same species, e.g. *C. intestinalis*. Ascidians feed by pumping water through a mucus net, being continually produced, it traps the suspended particles. The water enters through the inhalant siphon, which is lined with cilia that pumps the water through the mucus net. The water exits through the exhalant siphon. The trapped particles from the mucus net is rolled into a food string and then drawn to the oesophagus. It is not clear if the cilia may sort particles, but when particles have entered through the inhalant siphon and into the pharynx they will subsequently be trapped by the mucus net. (Petersen, 2007). *C. intestinalis* is an ectotherm and thereby more prone to environmental changes, which makes it a suitable model organism for studying the effects of global climate change (Melzner et al., 2009; Paaijmans et al., 2013). Most ascidians are very sensitive to disturbance, and it is not apparent by looking at the animal whether it is pumping at an optimal rate or not. Low clearance rates can therefore be a result of several suboptimal conditions for the model organism. It has also been debated whether some kind of physiological control could be involved (Petersen et al., 1999; Petersen & Riisgard, 1992).

Studying the ecological responses of ascidians has become very prominent work as they are the most common fouling organism on artificial structures like aquaculture gear, buoys, ropes, etc. Ascidians are a highly invasive species making coastal marine waters the most

invaded habitat on the planet. Ascidians have in fact become a global problem because of their quick dispersal, wide environmental tolerance, frequent population outbreaks and other economic and ecological impacts (Zhan et al., 2015). Their large numbers and potentially high filtration capacity makes them important inhabitants in coastal marine waters. *C. intestinalis* is actually considered a potential bioindicator species in coastal zones because of their resistance against pollutants (Caputi et al., 2015). Ascidians are often found in large populations which may generate large quantities of deposits, like feces. These deposits will in turn provide a great source of nutrition that many benthic organisms rely on for their own survival (Qi et al., 2015). With all this in mind, it is considered to be of utmost importance to further investigate how benthic suspension feeders will react to multiple factors, as the future effects of global climate change will occur with multiple stressors concurrently (Byrne & Przeslawski, 2013; Doney et al., 2009; Doney et al., 2012)

2.0 Materials and Methods

2.1 Sampling and preparation

For both the temperature experiment (Exp. 1) and the combined ocean warming (OW) and ocean acidification (OA) experiment (Exp. 2) the research animal was the tunicate species *Ciona intestinalis*. In Exp. 1 the animals were collected in September at 11-13 °C and in Exp. 2 they were collected in December at 11-12 °C. Individuals of the tunicate were collected over two days at two localities on the Institute of Marine Research (IMR) research facilities in Austevoll. The two localities were on two floating docks where the animals were collected by gently picking them off their attachment site. The animals were found right below the surface attached to the docks, rope or small buoys next to the docks. The animals were mainly within a size range of 10-20 mm in length, and were kept in a container with flow-through seawater. After collecting enough animals they were assessed for the right size again and viable individuals were glued onto small squares of Velcro straps. This was done by placing a small drop of Casco Express Glue on the Velcro and gently pressing the animals' holdfast against it. The animals were out of the water for 15-20 seconds while being glued before they were placed in a bucket with fresh seawater. After twenty tunicates had been glued on Velcro they were placed in a 1000 L tank to acclimatize and the seawater in the bucket was exchanged before another twenty animals were glued on Velcro. The small pieces of Velcro with tunicates attached were put on long straps of corresponding Velcro hanging vertically in the 1000 L tank. After all the tunicates were placed in the 1000 L tank they were left to acclimatize for 4-5 days, depending on when they were put into the tank. The tank had fresh seawater continuously being pumped in from right outside the research facilities at a depth of 7 m.

Next I will describe the experimental design, execution and measurements of both experiments separately. Exp. 1 was my main experiment executed by me and my main supervisor, while Exp. 2 was mainly executed by my main supervisor. I was present in the beginning with setting up some of the experimental design in order to analyze and use the data resulting from Exp. 2.

2.2 Experimental design

2.2.1 Temperature experiment (Exp. 1)

After acclimatization an equal number of animals were placed in small tanks in a naturally fed and a food limited set-up. Both set-ups had 4 different temperature treatments at 7 °C, 12 °C, 15 °C and 17 °C where each of these temperature treatments comprised 3 tanks each. This leaves a total of 12 tanks in each set-up (Figure 2.2.1). These 12 animal tanks in the fed set-up received unfiltered water while the food limited set-up of another 12 animal tanks received filtered water. The fed set-up was placed inside a container which cooled down the air temperature to 6°C. Sea water was pumped in from a depth of 7 m to a 1000 L tank which distributed water further to 4 header tanks placed on the floor. These four header tanks were called tank 1-4, one for each temperature treatment, and distributed water further to the 12 animal tanks. Tank 1 distributed water to the three 7°C tanks (1A, 1B, 1C), tank 2 distributed water to the three 12°C tanks (2A, 2B, 2C), tank 3 distributed water to the three 15°C tanks (3A 3B, 3C), and tank 4 distributed water to the three 17°C tanks (4A, 4B, 4C). There were heaters in tank 2, 3 and 4 to warm up the seawater for it to come out at the right temperature in the animal-tanks. Tank 2 and 3 had one heater each while tank 4 had two heaters as it was the warmest treatment. There were no heaters in tank 1 since the nominal temperature were supposed to be lower than the sea surface temperature actually was. The water from the four header tanks were pumped further to four new header tanks placed right below the ceiling above the 12 animal tanks. The water from tank 1 was led in metal-pipes and hoses along the metal floor for the water-temperature to drop down before it was led to the new header tank. From these 4 new header tanks the water was led to the 12 animal tanks. The water flow into the 12 animal tanks was regulated by membrane flow-meters set at 35 L/H. The hoses leading the water from header tank 3 and 4 were insulated the whole way through to the animal tanks, including insulation of the new header tanks below the ceiling and the animal tanks itself. To sufficiently warm up the water and maintain the temperature in tank 3A-C and 4A-C to respectively 15°C and 17°C, a small heater were placed in each of these tanks. All header tanks and animal tanks had an over-flow system. Since the treatment tanks warming up the water before it were led to the animal tanks showed to have no effect on the animals growth rate ($F_{1, 173} = 0.837$, $P=0.362$) they were removed from the model.

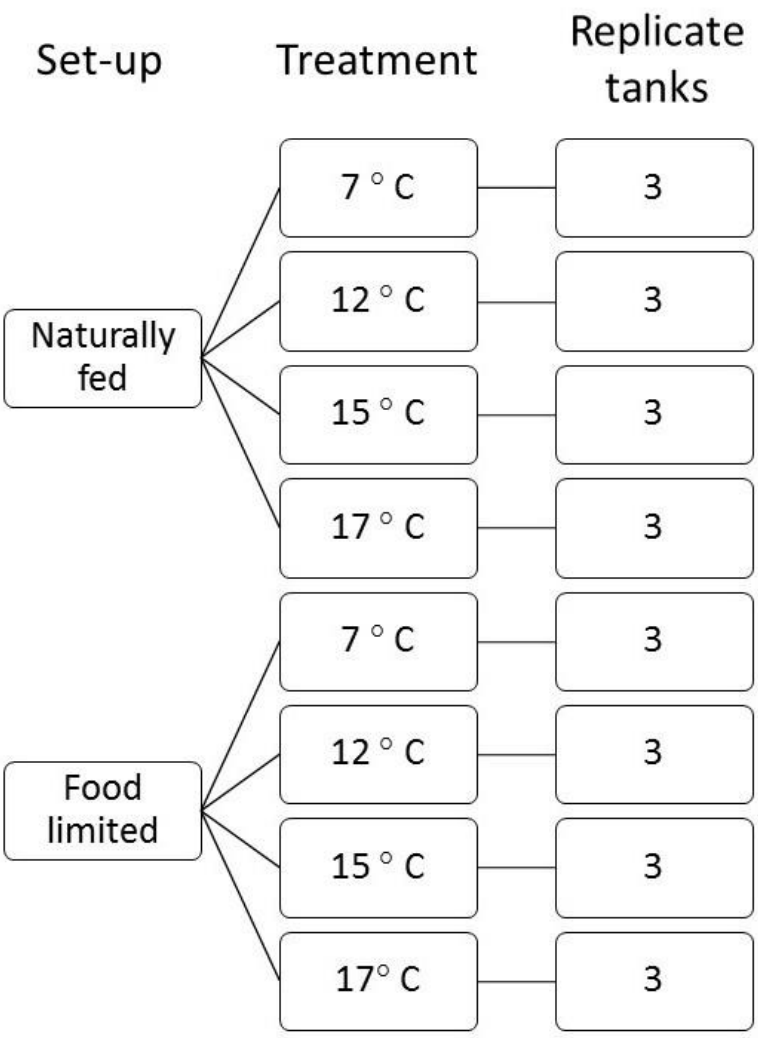

Figure 2.2.1: Experimental design of Exp. 1

In the food limited set-up with filtered seawater there were three taps with 6 °C, 12 °C and 15 °C seawater leading to the animal tanks. This sea water was pumped up from a depth of 160 m outside the research facilities and was pre-heated before coming out the three taps. From the 6 °C and 12 °C tap the water were led in each hose through each set of two filters with 10 and 0.2 µm mesh size. From the 15 °C tap the water were led in two hoses through each set of two filters with the same mesh size. After being led through these 4 sets of filters each of the four hoses with seawater went to a Gardena water distributor leading the water into three animal tanks each. The 6 °C water came out as 7 °C in animal tank B10, B11 and B12 and the 12 °C water came out as 12 °C in animal tank B4, B5 and B6. The water from one of the 15 °C hoses came out as 15 °C in animal tank B1, B2 and B3. The water from the other

15 °C hose came out in tank B7, B8 and B9. In these three tanks (B7, B8 and B9) heaters were used to warm up the water to 17 °C. Insulation was also put around the three animal tanks to help maintain the warm 17 °C water. The air temperature in the room of this set up was 13-14 °C. Both the fed and the food limited set-up had the same light regime with natural lighting placed right above the animal tanks with a translucent plate between the lights and the animal tanks. The lights were controlled by timers set at 12 hours on and 12 hours off.

2.2.2 Combined OW and OA experiment (Exp. 2)

After acclimatization the animals were placed in a naturally fed and food limited set-up of 12 animal tanks each. The experimental set-up and tank limitations were the same as in Exp. 1, however, with 4 different treatments of combined temperature and pH limitations (Figure 2.2.2). Two temperature treatments were chosen after assessing the results from Exp. 1 along with two pH treatments. The two set-ups thus had 4 different treatments at 12 °C with pH 8, 12 °C with pH 7.7, 15 °C with pH 8 and 15 °C with pH 7.7 where each of these treatments comprised 3 tanks each. The temperature in each animal tank were stabilized and under control before the pH was modified in the header tanks for each of the 4 treatments. The pH was monitored individually for each treatment and the effect of temperature, salinity and total alkalinity were accounted for. To achieve the two chosen pH levels CO_2 was added to the water using a digital multi-parameter transmitter (Endress & Hauser, Liquiline CM448).

To determine the clearance rate of particles the animals were placed in the same flow through system and feeding chambers as described by Strohmeier et al., (2009). The internal dimensions of these feeding chambers had a 10.5 cm width, 22 cm length and 10 cm height. The chambers are designed with the purpose of restricting recirculation and hence inhibit re-filtration of particles. In addition the water flow rate was kept at a level of 6 L/h^{-1} which also inhibits re-filtration of particles (Strohmeier et al., 2009).

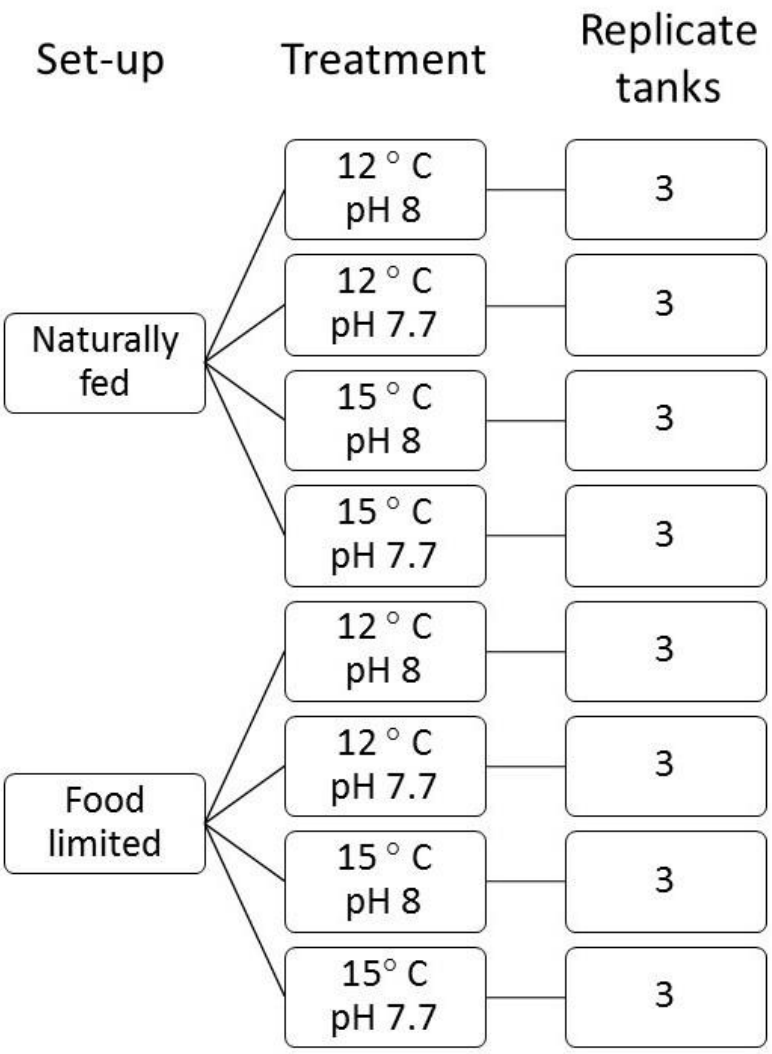

Figure 2.2.1: Experimental design of Exp. 2

2.3 Execution

2.3.1 Temperature experiment (Exp. 1)

At the project start the animals were taken out of the acclimatization tank ten at a time in a small bucket with fresh seawater. The animals were assessed consecutively and if found viable they were weighed with the Velcro and placed directly on vertical strips of Velcro in the animal tanks. Viability was determined on the animals' appearance and if it looked healthy and alive. Ten animals were placed on two strips of Velcro in each tank, which means a total of 240 animals were placed in the animal tanks at project start. The animals were then monitored over the next 4 weeks. In the fed set-up, a particle counter (PAMAS

GmbH, Model S4031GO) performed continuous monitoring of the particle content. The PAMAS was set up to run three rounds in a half hour. The water samples it ran were controlled by four pumps pumping water from the four treatments one at a time. These pumps were controlled by timers to pump a half hour each through the day. This system made sure that the PAMAS had run samples of each of the four different treatments after two hours had gone before it started over again. The air temperature and water temperature in each animal tank of both the fed and food limited set up were measured twice a day and the length of each animal was measured twice a week on set days. Feces and biofouling were also removed twice a week in the food limited set-up. Checking the filters and running water samples from the hose of each of the four treatments through the PAMAS were done twice a week to monitor the particle content in the food limited set-up.

2.3.2 Combined OW and OA experiment (Exp. 2)

At project start animals were assessed and placed in the animal tanks in the same manner as of Exp. 1 with ten animals in each animal tank. The animals were then monitored over the next 4 weeks in the same manner as described for Exp. 1 regarding monitoring of particle content, temperature measurements, length measurements, removal of feces and biofouling, checking filters and running water samples through the PAMAS. In this experiment, the pH was also measured twice a day for each animal tank and pCO_2 were calculated from this.

2.4 Measurements

2.4.1 Temperature experiment (Exp. 1)

After 4 weeks the experiment was brought to an end. Ten O_2-bottles of 100 ml were used to make the final O_2 measurements and oxygen-sensitive dots were glued on the inside of each bottle 12 hours before usage. Velcro to attach the animals was also glued inside each bottle. Nine animals were chosen from each treatment in both the fed and food limited set-up and placed one at a time inside nine of the O_2-bottles leaving O_2-bottle No. 10 as the control. The O_2-bottles were then consecutively connected to the O_2-meter and placed in a separate tank

with equivalent conditions regarding temperature and pH as the current treatment. Animals from the 12 °C treatment in the food limited set-up were left in the O_2-bottles for one hour with the connected pump on, pumping water through the bottles, and then left for a two hour incubation time with the pump off. The O_2-meter was continuously taking measurements during this time using optical oxygen probes (PreSense, Oxy-10 mini, 10 channel fiber optic oxygen transmitter; vernier system, labquest). The O_2-meter will measure the fluorescent energy being released by the oxygen-sensitive dots that were glued inside each of the ten O_2-bottles. The changes in fluorescence are detected by a fluorimeter attached outside the O_2-bottle parallel to the oxygen-sensitive dot. To control the system and collection of data fiber optic cables were connected to a PC. The final O_2-measurements were taken immediately after the two hour incubation time had passed. Afterwards the nine animals were taken out one at a time and measurements were taken for length, width, mass and volume. The length and width of each animal was measured first with a caliper after gently being lifted out of the water making sure the animal did not retract itself. The animals were then weighed on a scale with the piece of Velcro they had been attached to before they were peeled carefully off the Velcro and weighed alone. Lastly, the animal volume was measured by putting them into a small measuring beaker containing an exact amount of water where the difference in mL was measured after the animal was put in. These measurements were done for all treatments in both the fed and the food limited set-up in both experiments. The animals were left in the O_2-bottles for 30 minutes with the pump on and two hours with the pump off, before taking the final measurements described. After all the O_2-measurements were done on the selected animals in all the treatments, the rest of the animals left in the animal tanks were taken out one at a time and measured for length, width, mass and volume like described.

2.4.2 Combined OW and OA experiment (Exp. 2)

After 4 weeks the experiment was brought to an end. The O_2 measurements was taken in the same manner as described in Exp. 1 using 9 animals from each treatment in both set-ups (naturally fed and food limited). In addition the pH was measured instantly after the two hour incubation period within 30 seconds of opening each chamber. These pH measurements were together with salinity, temperature and total alkalinity used to calculate

pCO_2. The clearance rates of 9 animals from each treatment in both set-ups were also measured at the end of Exp. 2. The animals were placed in the feeding chambers and left for 1 hour to resume their feeding activity prior to sampling. Water samples were collected from the outflow of each feeding chamber and the concentration of suspended particles was measured using the PAMAS and the water sample was then filtered to find the particulate organic matter (POM). The flow rate through each chamber was also recorded. Finally all the animals of Exp. 2 were taken out one at a time and measured for length, width, mass and volume like described in Exp. 1.

The following calculations were made to estimate the clearance rate, absorption efficiency, energy ingestion, energy absorption, respiration rate (energy respired) and the scope for growth by using the following formulas:

$$\textit{Clearance rate (L } h^{-1}\textit{) = Flow rate (L } h^{-1}\textit{) x } (C_1 - C_0)/C_1$$

Where C_1 is the mean particle concentration between 1.5-15 µm from the controls, and C_0 is the particle concentration between 1.5-15 µm from each animal coming out of the flow-through chambers.

$$\textit{Absorption efficiency} = (F - E)/[(1 - E)F]$$

Where F = ash-free dry weight:dry weight ratio of food, and E = ash-free dry weight:dry weight ratio of feces.

$$\textit{Energy ingestion = Maximum clearance rate (L } g^{-1}\ h^{-1}\textit{) x (mg POM } L^{-1}\textit{) x (23 J } mg^{-1}\textit{ POM)}$$

Where the energy content of POM is c. 23 J mg^{-1} ash-free dry weight.

$$\textit{Energy absorption = Energy ingestion x Absorption efficiency}$$

$$\textit{Energy respired = (µmoles } O_2\ g^{-1}\ h^{-1}\textit{) x 0.456}$$

Where the heat equivalent of oxygen uptake is 0.456 J μmol^{-1} O_2.

$$\textit{Scope for growth = Energy absorption – Energy respired}$$

These calculation formulas are all retrieved from “Practical Procedures for the measurement of scope for growth” (Widdows & Staff, 1997).

2.5 Statistical analysis

Generalized linear mixed models (GLMM) were used to compare O_2, clearance rate, energy ingestion, absorption efficiency, energy absorption, scope for growth and growth between pH, temperature and feeding set-up. When calculating the O_2 uptake, mass was added as a covariant, so was the tank effect, but neither had any effect on the O_2 uptake, it was therefore removed from the model. Estimated marginal means (EMM) was then used to test the significant differences between different combinations of the factors (pH, temperature, feeding set-up). F-tests were generated for detection of any significant differences between these combinations of factors. All the statistical analysis performed was done using SPSS software v.22 (SPSS, Chicago, IL, USA). All the values are presented as means ±s.e.m.

3.0 Results

3.1 Oxygen uptake

In general the oxygen uptake increased with temperature in Exp. 1. This response did however show a significant difference between the naturally fed animals and the food limited animals ($f_{3, 63}$=24.527, p<0.001). The naturally fed animals increased their MO_2 (MO_2; mass adjusted) from 0.497±0.172 µmol h^{-1} at 7 °C to 3.449±0.172 µmol h^{-1} at 15 °C before showing a decrease at 17 °C to 2.129±0.172 µmol h^{-1}. The food limited animals however, showed a continuous increase from 0.01±0.192 µmol h^{-1} at 7 °C to 1.379±0.190 µmol h^{-1} at 17 °C (Figure 3.1.1). Among these temperatures the food limited animals had the lowest MO_2 ($f_{1, 63}$=182.574, p<0.001).

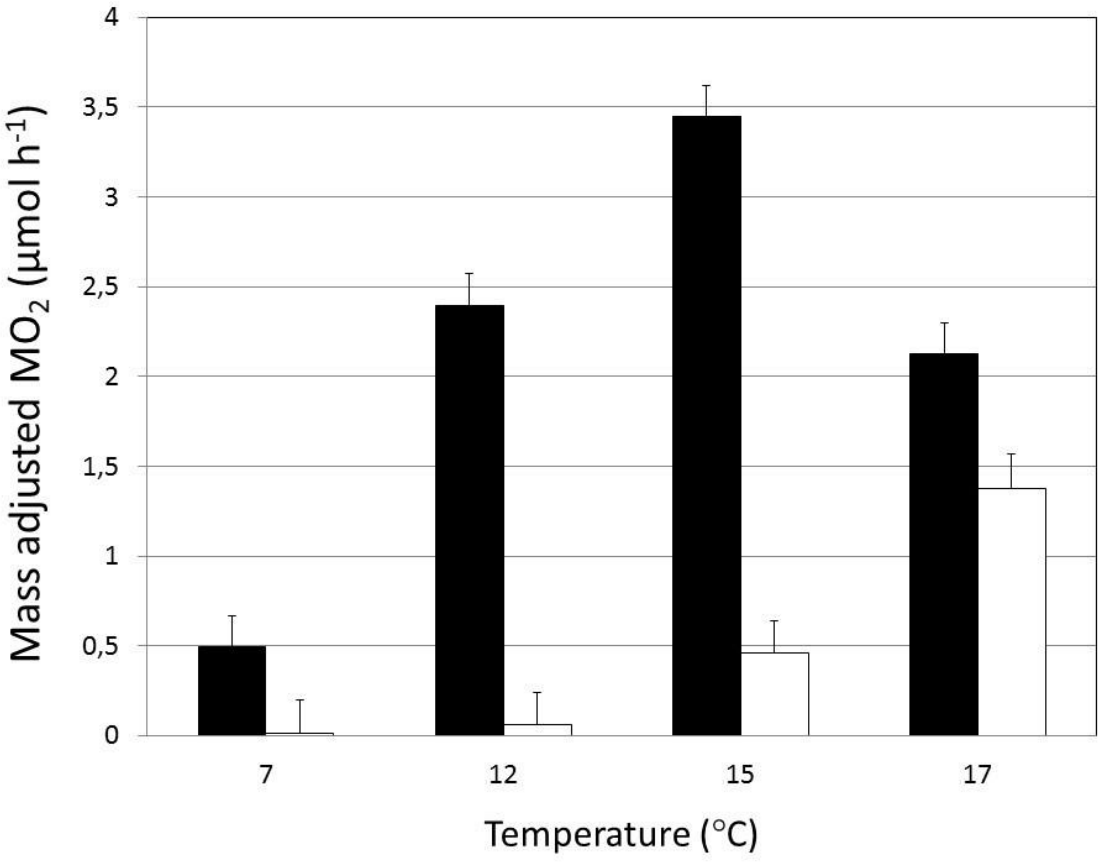

Figure 3.1.1: MO_2 response in *Ciona intestinalis* after 4 weeks of exposure to different temperatures adjusted to mean individual mass (0.81 g) between naturally fed animals (black) and food limited animals (white) in Exp. 1.

The animals in Exp. 2 had an overall lower MO_2 response compared to the animals in Exp. 1. The MO_2 response in Exp. 2 was also significantly lower in the food limited animals ($f_{1, 63}$=16.330, p<0.001) compared to the naturally fed. The MO_2 was highest in naturally fed animals at ambient pCO_2 (380 µatm) and elevated temperature (15 °C) (1.379±0.112 µmol h^{-1}) and was lowest in the food limited animals (0.621±0.115 µmol h^{-1} to 0.778±0.112 µmol h^{-1}, Figure 3.1.2). The MO_2 response in food limited animals did not significantly vary between pCO_2 ($f_{1, 31}$=2.556, p=0.120) or temperature ($f_{1, 31}$=0.435, p=0.514). In the naturally fed animals there is a significant reduction in MO_2 in the elevated pCO_2 (750 µatm) and elevated temperature treatment (mean difference = 0.595 µmol h^{-1}; $f_{1, 31}$ = 17.521, p<0.001; Figure 3.1.2).

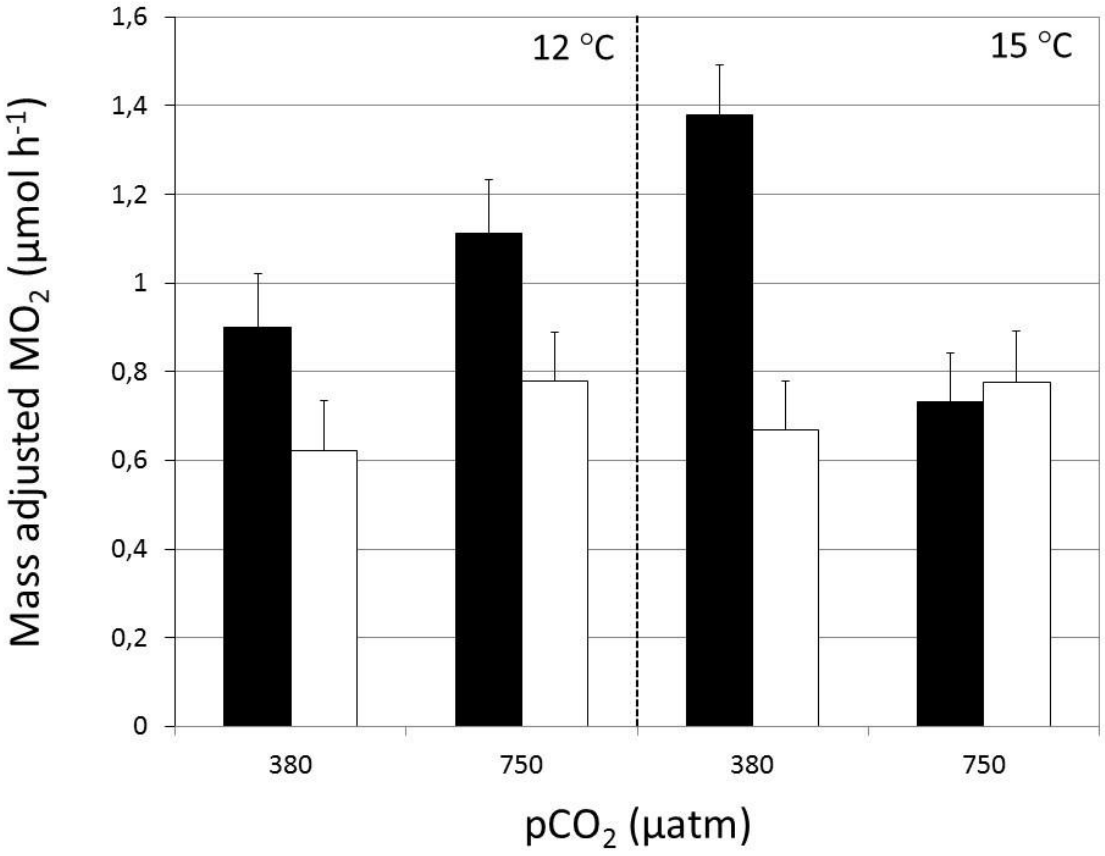

Figure 3.1.2: MO_2 response adjusted to mean individual mass (2.6 g) in *Ciona intestinalis* after 4 weeks of exposure to pCO_2 and selected temperatures between naturally fed animals (black) and food limited animals (white) in Exp. 2.

3.2 Clearance rates

In Exp. 2 with combined OA and OW, the naturally fed animals had the lowest clearance rates (CR; adjusted for partial size) at ambient pCO_2 and elevated temperature (1.783±1.097 ml min^{-1}). The food limited animals had the highest CR at ambient pCO_2 in both temperature treatments (12 °C, 31.861±1.343 ml min^{-1}, 15 °C, 33.743±1.244 ml min^{-1}; Figure 3.2.1). Except for the elevated pCO_2 and elevated temperature treatment, the CR is significantly higher in the food limited animals ($f_{1, 1079}$=321.476, p<0.001). CR were significantly reduced in the food limited animals at elevated pCO_2 in both temperature treatments (12 °C, $f_{1, 1079}$ = 27.696, p<0.001; 15 °C, $f_{1, 1079}$ = 270.439, p<0.001; Figure 3.2.1), although the reduction was greater at the elevated temperature treatment (mean difference; 12 °C, 9.999±1.900 ml min^{-1}, 15 °C, 27.272±1.658 ml min^{-1}). CR were significantly reduced in the naturally fed animals in the elevated pCO_2 at ambient temperature (12 °C) (mean difference = 13.758±1.551 ml min^{-1}; $f_{1, 1079}$ = 78.661, p<0.001), in contrast to the naturally fed animals in elevated pCO_2 at elevated temperature where the CR were significantly higher (mean difference = 4.688±1.551 ml min^{-1}; $f_{1, 1079}$ = 9.134, p<0.01).

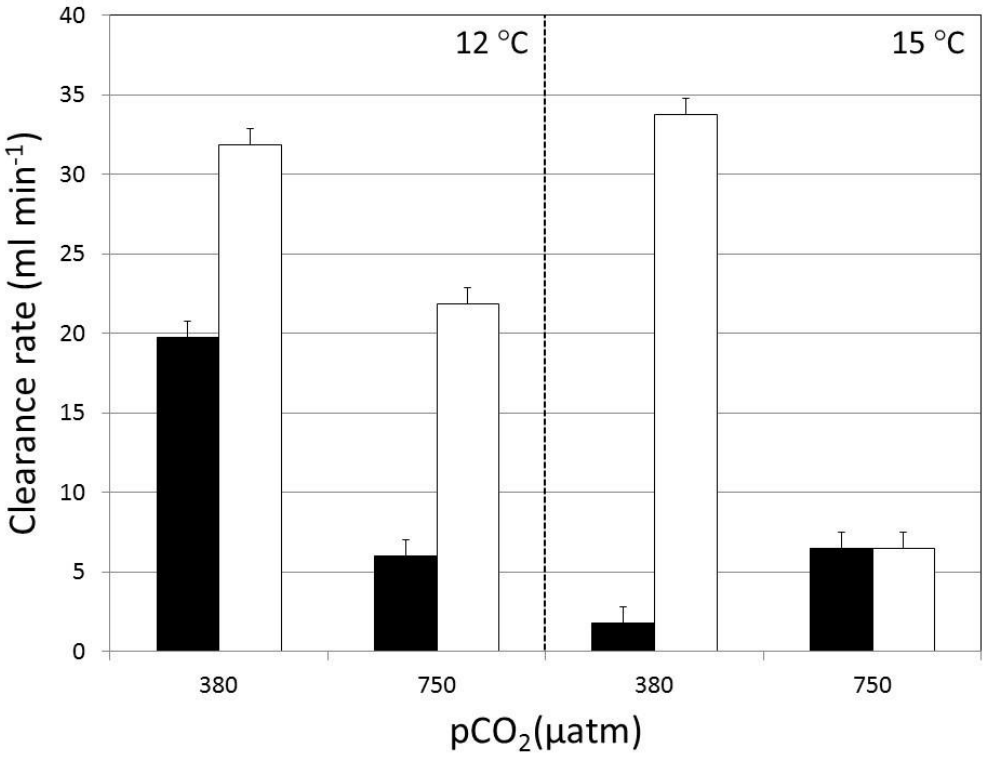

Figure 3.2.1: Clearance rates response in *Ciona intestinalis* after 4 weeks of exposure to pCO_2 and selected temperatures between naturally fed animals (black) and food limited animals (white) in Exp. 2.

3.3 Energy ingestion and absorption through feeding

The energy ingested (adjusted for mass) in Exp. 2 had overall higher values in the food limited treatments, with the exception of the elevated pCO_2 and temperature treatment, just like the CR ($f_{1, 52}$=9.402, p<0.01, Figure 3.3.1). The energy ingestion in the naturally fed animals was reduced in comparison with controls in all elevated pCO_2 and/or temperature treatments. The food limited animals however only had reduced energy ingestion in the elevated pCO_2 and temperature treatment ($f_{1, 52}$ = 10.183, p<0.01, Figure 3.3.1). The overall absorption efficiency also showed the same pattern as CR and energy ingestion, with significant higher absorption efficiency values in the food limited animals compared to the naturally fed animals ($f_{1, 59}$=7.697, p<0.01).

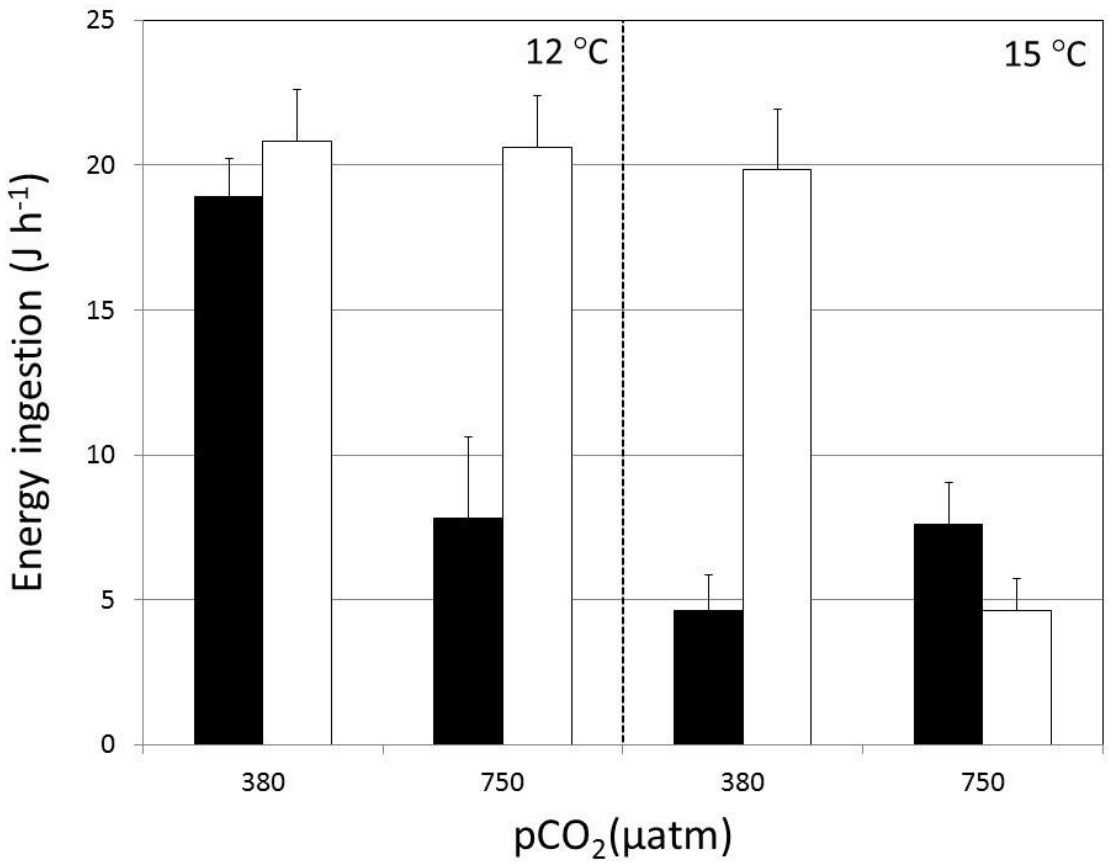

Figure 3.3.1: Energy ingestion response in Ciona intestinalis after 4 weeks of exposure to pCO_2 and selected temperatures between naturally fed animals (black) and food limited animals (white) in Exp. 2.

These higher levels of absorption efficiency were reduced when the animals were in the elevated pCO_2 and temperature treatment, as observed in CR and energy ingestion. This resulted in a significant interaction between food limitation, temperature and pCO_2 ($f_{1, 59}$ = 5.589, $p<0.05$). The effects from these interactions on energy ingestion and absorption resulted in the food limited animals showing higher energy absorption rates than the naturally fed in every treatment except the elevated pCO_2 and temperature treatment (ambient pCO_2 and temperature, $f_{1, 48}$ = 4.451, $p<0.05$; elevated temperature, $f_{1, 48}$ = 7.346, $p<0.01$; elevated pCO_2, $f_{1, 48}$ = 19.239, $p<0.001$, combined elevated pCO_2 and temperature, $f._{1, 48}$ = 0.271, $p=0.605$, Figure 3.3.2). In naturally fed animals the mean energy absorption values were highest at ambient pCO_2 and temperature, and were negative in every other elevated temperature and/or pH treatments. In food limited animals the only negative mean energy absorption was observed in the elevated pCO_2 and temperature treatments, here the animals showed a significantly lower energy absorption than the animals that were exposed to just elevate temperature and ambient pCO_2 ($f_{1, 48}$ = 8.809, $p<0.01$) or pCO_2 ($f_{1, 48}$ = 9.064, $p<0.01$) in isolation did (Figure 3.3.2).

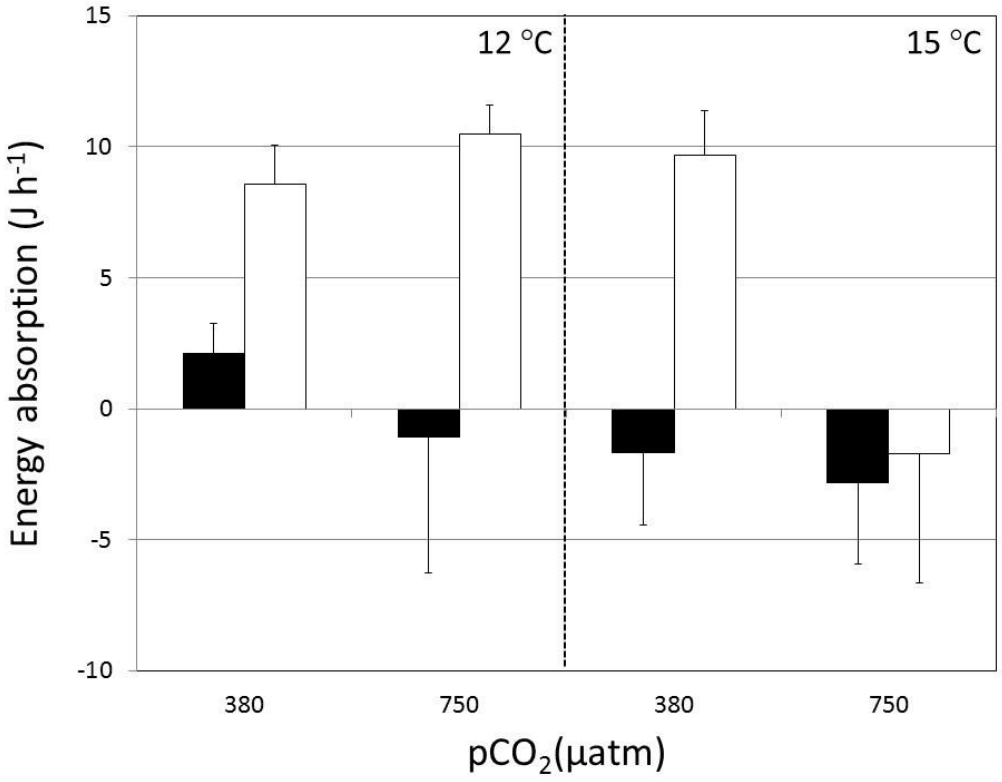

Figure 3.3.2: Energy absorption response in *Ciona intestinalis* after 4 weeks of exposure to pCO_2 and selected temperatures between naturally fed animals (black) and food limited animals (white) in Exp. 2.

3.4 Scope for growth

The response for scope for growth showed a significant difference between the naturally fed animals and the food limited animals ($f_{1, 64}$ =19.403, p<0.001). There was also a significant interaction with temperature and pCO_2 ($f_{1, 64}$=4.258, p<0.05). The naturally fed animals decreased continually from the ambient pCO_2 at 12 °C (1.417±1.578 J h^{-1} g^{-1}) to the elevated pCO_2 at 15 °C (-3.171±5.538 J h^{-1} g^{-1}, Figure 3.4.1). The food limited animals had a generally higher response than the naturally fed, with the highest response in elevated pCO_2 at 12 °C (5.479±1.595 J h^{-1} g^{-1}, Figure 3.4.1). For the food limited animals, scope for growth showed a significant variation with temperature ($f_{1, 64}$=8.185, p<0.01) and pCO_2 ($f_{1, 64}$=12.797, p<0.001) at the elevated pCO_2 and temperature treatment.

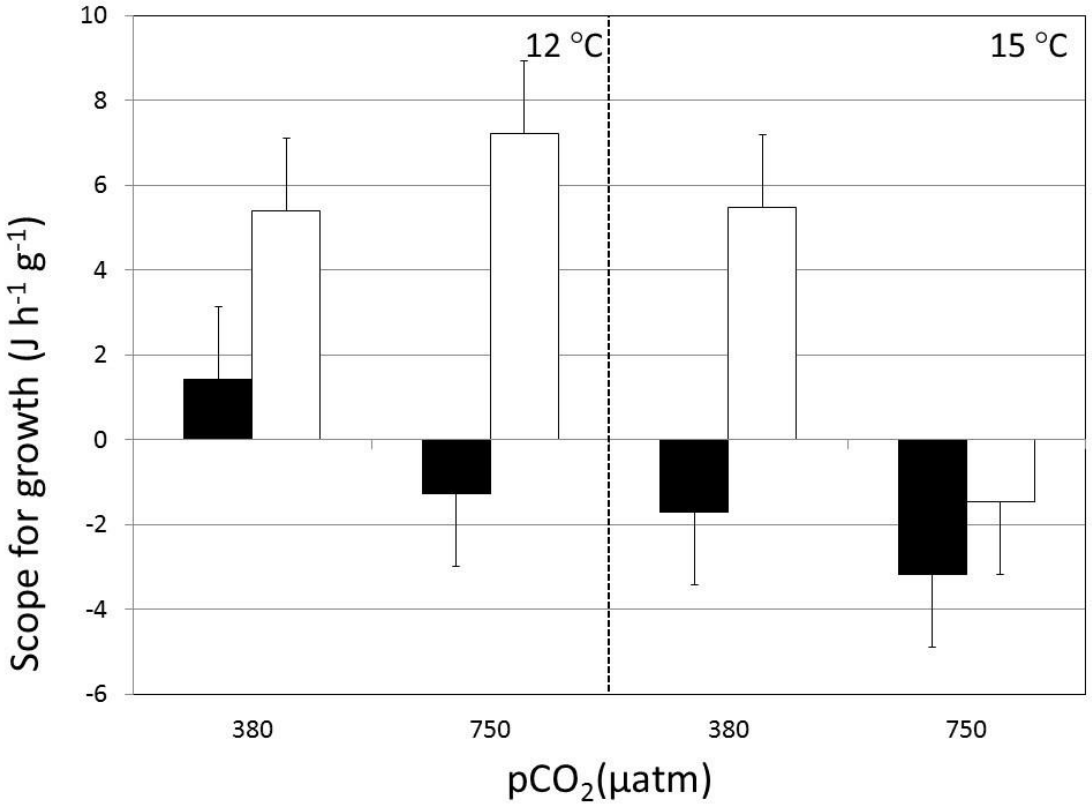

Figure 3.4.1: Scope for growth response in *Ciona intestinalis* after 4 weeks of exposure to pCO_2 and selected temperatures between naturally fed animals (black) and food limited animals (white) in Exp. 2.

3.5. Growth

In Exp. 1 the animals' change in length over time was significantly lower in the food limited individuals ($f_{1, 174} = 65.824$, $P<0.001$). The food limited animals at 12 °C, 15 °C and 17 °C actually had a negative growth. The naturally fed animals at 12 °C had the highest growth rate, while the food limited animals at the higher temperatures of 15 °C and 17 °C showed the lowest negative growth rate (Figure 3.5.1). The temperature treatment had no significant effect on growth in naturally fed animals ($f_{3, 174} = 1.863$, $P=0.138$), unlike the food limited animal which had a significantly lower growth at 15 °C and 17 °C ($f_{3, 174} = 5.922$, $p<0.001$).

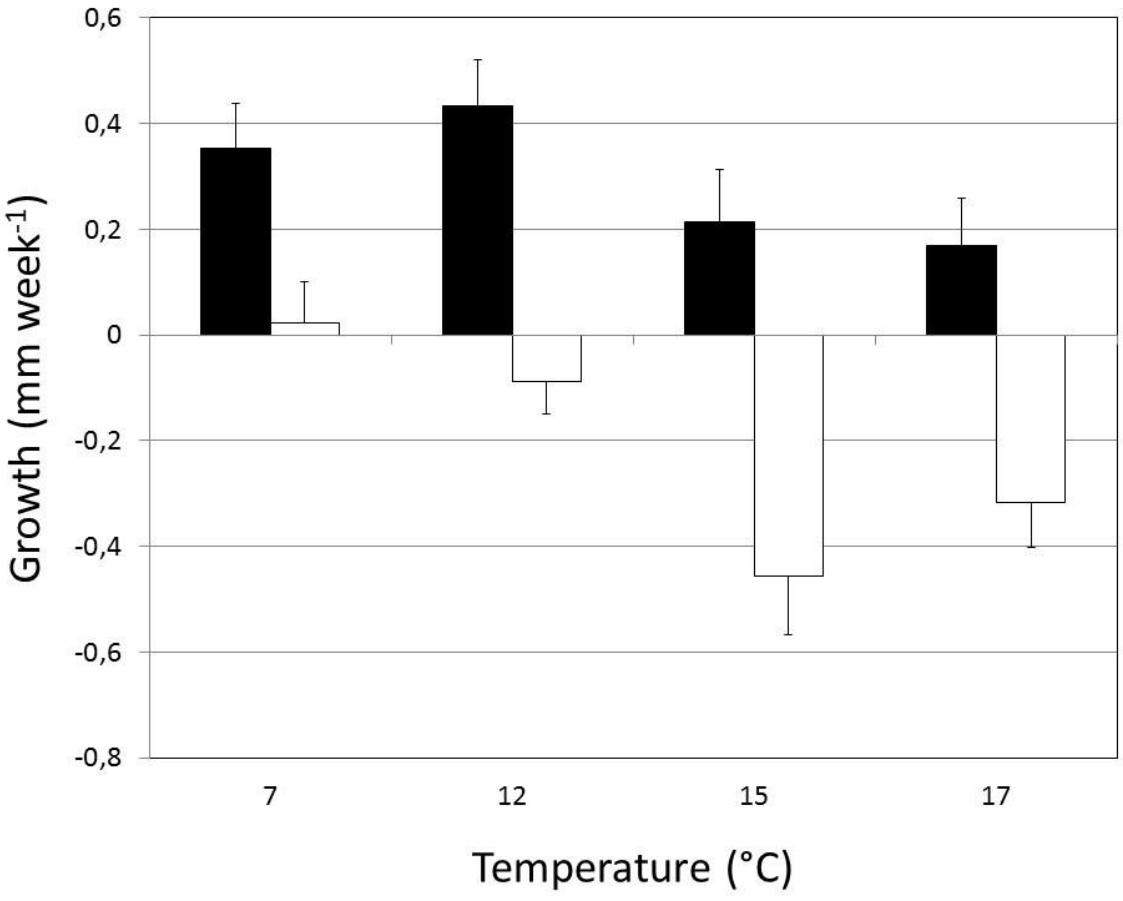

Figure 3.5.1: Growth response in *Ciona intestinalis* after 4 weeks of exposure to the four different temperature treatments between naturally fed animals (black) and food limited animals (white) in Exp. 1.

In Exp. 2 with combined OA and OW, the highest growth rate was seen in the naturally fed animals in the ambient pCO_2 and 12 °C incubation (0.658±0.693 mm $week^{-1}$). The animals in the other three incubations all showed a negative growth which ranged from -4.776±0.705 mm $week^{-1}$ to -0.961±0.669 mm $week^{-1}$ (Figure 3.5.2). In general, growth was lower in the food limited animals ($f_{1\ 222}$ = 12.128, p<0.001) and did not significantly vary between temperature ($f_{1,\ 112}$=1.660, p=0.200) or pCO_2 ($f_{1,\ 112}$=0.704, p=0.403) treatments. The growth of the naturally fed animals was however significantly lower at the elevated temperature in both ambient (mean difference = 3.816±0.972 mm $week^{-1}$; $f_{1,\ 222}$ = 15.404, p<0.001) and elevated pCO_2 treatments (mean difference = 4.356±0.971 mm $week^{-1}$; $f_{1,\ 222}$ = 20.128, p<0.001).

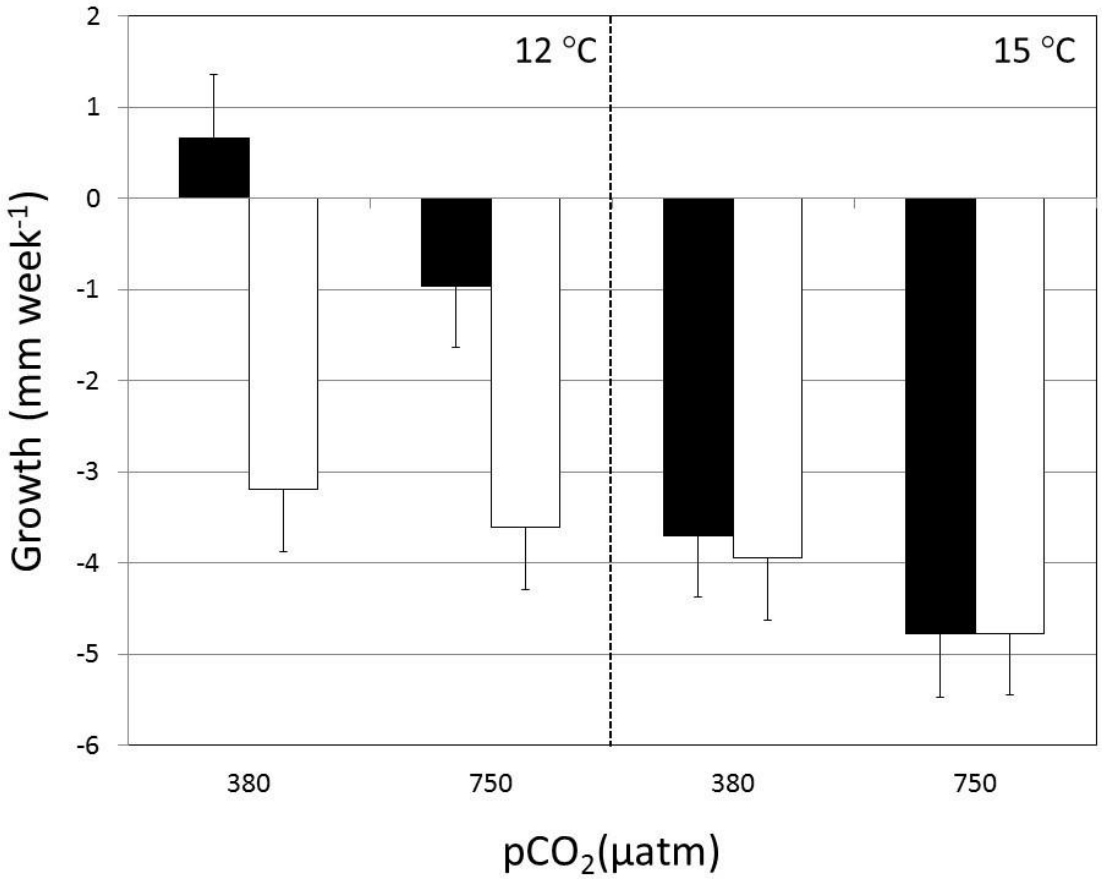

Figure 3.5.2: Growth response in *Ciona intestinalis* after 4 weeks of exposure to pCO_2 and selected temperatures between naturally fed animals (black) and food limited animals (white) in Exp. 2.

4.0 Discussion

4.1 Metabolic rate

Data from Exp. 1 showed a significant difference in the MO_2 response between the naturally fed and the food limited treatments. The MO_2 response in naturally fed animals gradually increased from 7 °C up to 15 °C before decreasing at 17 °C (Figure 3.1.1.), which could indicate that they have reached their optimum temperature range between 12-15 °C. The optimum temperature range of an animal is at the temperatures where the organisms' performance in e.g. growth, reproduction, foraging, immune competence, behaviour and competitiveness are at its optimum (Portner & Farrell, 2008). The organisms' performance is dependent on the aerobic scope (AS), which is the increasing oxygen consumption rate from standard to maximum (Auer et al., 2015). This means that their maximum aerobic threshold in this experiment could be different from the optimum temperature range, as it is where the AS is at its maximum and this is not necessarily, where the animal is at its optimum. This could really be somewhere between 12-17 °C, but it is difficult to be more accurate since our experiment did not include any temperatures in between. Performance can fall below their optimum temperature range during cooling and warming. This happens because at their lower and upper pejus temperatures the oxygen capacity is limiting and will cause hypoxia. These pejus temperatures sets the thermal limitations of the animals (its thermal window) and when animals fall beyond these critical temperatures they can only exist in a passive, anaerobic state (Portner & Farrell, 2008). In contrast, the food limited animals showed a steady increase in their MO_2 response at 7 °C through to 17 °C. From the data in this experiment, it is not possible to see what the optimum temperature is for the food limited animals since the MO_2 response seems to increase gradually with each higher temperature treatment. What can be said is that the temperature of the maximum aerobic threshold for food limited animals is probably higher than the temperature treatments used in this experiment, and therefore higher than for the naturally fed animals. This indicates that *C. intestinalis* has a higher thermal tolerance level when the access to food is low (Portner & Farrell, 2008). With low food availability, the animals' metabolic rate might decrease in order to survive (Auer et al., 2015). This lowers their oxygen demand and further decrease certain metabolic processes, e.g. growth. These physiological changes might allow the animals to

better maintain its performance in a stressed state when the food availability is low (Auer et al., 2015), which might explain why they show a higher maximum aerobic threshold.

In the future, the climate is expected to change with the prediction of a drop in pH by 0.3-0.4 units as well as an 2-3 °C increase in sea surface temperature (Mackenzie et al., 2014). In Exp. 2 with combined ocean warming (OW) and ocean acidification (OA) the MO_2 response were also different between the naturally fed and the food limited animals (Figure 3.1.2). The MO_2 response in food limited animals did not vary between the different pCO_2 and temperature treatments, which indicates that these animals might be coping with their surroundings. However, the naturally fed animals seem to tolerate the lower pH and the higher temperature separately, but the combination of the elevated temperature and the lower pH results in the lowest MO_2 response among the naturally fed. This indicates that they have reached their thermal limit at 15 °C and ambient pCO_2 . This conform with the result in Exp. 1 where the naturally fed animals also seem to have their maximum aerobic threshold at 15 °C (Figure 3.1.1). The difference in metabolic rate was almost twice as high in the first temperature experiment (Exp. 1) as it was in the combined OW and OA experiment (Exp. 2), although the conditions were the same at ambient pCO_2 in both temperature treatments in Exp. 2 as they were in Exp. 1 at 12 °C and 15 °C. The reason for this could be dependent on season as the two experiments, and hence animal collection, were conducted at different times of the year (Urbina & Glover, 2013). There are several physiological processes that make up the metabolic rate, which can be influenced by a great number of intrinsic and extrinsic factors (Glazier, 2005). An important intrinsic factor is the body mass of an organism (Urbina & Glover, 2013). It has been suggested in previous studies on fish (Urbina & Glover, 2013) and insects (Verberk & Bilton, 2011) that different strategies for oxygen uptake and its efficiency might have an impact on metabolic rate. How effective the oxygen uptake is might differ with body mass. The animals in the combined OW and OA experiment (Exp. 2) were generally larger in mass (2.6 g) and had a lower metabolic rate than the smaller animals in Exp. 1 (0.81 g). Metabolic rate can hence be dependent on both environmental oxygen available as well as body size (Urbina & Glover, 2013).

Seasonal changes might be an extrinsic factor that could have an impact on the metabolic rate since the animals of the two experiments where gathered 2 month apart. Exp. 1 was conducted first and the animals were hence gathered in end of September, in contrast to the

animals in Exp. 2 with combined OW and OA that were gathered in December. The sea temperatures are usually colder later in the year, which should have led to a larger mean mass for the animals collected in September compared to the animals collected in December. However this is not the case in our study since the temperatures were not that different. The supply of natural seston could be a possible explanation to difference in mean body mass of the two experiments. Natural seston is dependent on season, with a lower seston concentration during the winter season resulting when the primary production in the ocean is low (Velasco & Navarro, 2005). However, the animals collected in December for Exp. 2 had a generally larger mean mass (2.6 g) than the animals collected in September for Exp. 1 (0.81 g). A source of error could be that a different group of people collected the animals in the two sets of experiments, which might have led to a different mean size among the animals at the start of the two experiments. Environmentally available oxygen is another extrinsic factor that is particularly important in aquatic species as oxygen can vary considerably in different water bodies (Urbina & Glover, 2013). In lower temperatures the O_2 saturation will also be somewhat higher, since cold water are able to hold more dissolved O_2 than warmer water, which could have had an effect on the size of the collected animals in our experiments, since the sea temperature changes with season. As mentioned, body mass, temperature, environmental oxygen and different uptake strategies and effectiveness will together affect the metabolic rate and influence the results of this study.

4.2 Clearance rates

The clearance rate response (Figure 3.2.1) indicates that when ascidians are subject to low food concentrations they are more susceptible to changes in pH than temperature, and when they are under normal *ad libitum* conditions they are more likely to respond to changes in temperature. Both the naturally fed and the food limited animals had a reduced clearance rate when exposed to elevated pCO_2 and elevated temperature in combination, although the clearance rate is generally higher in the food limited animals compared to the naturally fed (Figure 3.2.1). This indicates that the food limited animals are able to upregulate clearance rate compared to the naturally fed, even when they are stressed by pCO_2 or temperature. However, they are unable to continue this when they are stressed by

both pCO_2 and temperature in combination. It has been discussed in previous studies whether suspension feeders might be able to physiologically regulate their clearance rate (Petersen & Riisgard, 1992; Riisgard & Goldson, 1997). Petersen et al. (1999) measured the beat frequency of the lateral cilia in the openings of the branchial sac in ascidians in order to see if they could in fact regulate their clearance rate. Beat frequency was seen to increase with increasing temperature at a low algal concentration and decrease with increasing algal concentrations at a constant temperature of 15 °C (Petersen et al., 1999), which conforms with the clearance rates for the food limited animals set against the fed animals in our study (Figure 3.2.1.). This variation in clearance rate as a response to particle concentrations has been interpreted in several manners, including it being a protective reaction to prevent an overload of the digestive system (Petersen & Riisgard, 1992) and it being a regulatory control of the filtration rate to maintain a constant ingestion (Navarro & Winter, 1982). This could make sense since the coastal waters where ascidians thrive are often prone to a high variety of particle concentrations and temperatures (Armsworthy et al., 2001). Clearance rate in *Ciona intestinalis* can hence be said to be a result of its beat frequency and change as a response to surrounding environmental factors, where two major factors have proven to be particle concentrations and temperature (Lisbjerg & Petersen, 2001; Petersen, 2007; Petersen et al., 1999). Clearance rate does however not consider particle size or whether it is organic and is therefore made up of all particles no matter how efficiently they are retained (Armsworthy et al., 2001). Ascidians are also believed to lack a particle sorting mechanism which is a disadvantage, and a potential source of error in this study, as it will affect the absorption efficiency and hence the energy absorption and growth of the animals (Petersen, 2007).

4.3 Energetics and scope for growth

Energy ingestion are linked to filtration rate, and hence to the clearance rate which we have already discussed. Both the energy ingested and the overall absorption efficiency in our study shows the same pattern as CR with the food limited animals showing the highest values compared to the naturally fed animals, the only exception is in the elevated pCO_2 and elevated temperature treatment (Figure 3.3.1). The significant interactions of food

limitation, temperature and pCO_2 shows effects which results in these generally higher energy ingestion and absorption rates for the food limited animals. This indicates that the food limited animals are able to upregulate their absorption efficiency when they are exposed to pCO_2 or temperature, compared to the naturally fed. However, they are not able to keep this up when they are stressed by both pCO_2 and temperature at the same time. This is evident when assessing the energy absorption rates of the food limited animals (Figure 3.3.2) where the only negative value is seen in the combined elevated pCO_2 and elevated temperature, which is significantly lower than the other treatments. For the naturally fed animals the mean energy absorption values were all negative except in the ambient pCO_2 and ambient temperature treatment, which means that they do not respond well to changes in either pCO_2 or temperature.

Our results corresponds to those of Navarro and Winter (1982) who found that the energy gain in the mussel *Mytilus chilensis* decreased with increasing food concentrations, and thus the highest energy gain was found among those in the lowest food concentrations. The energy absorbed by an animal is primarily utilized for standard metabolism, like growth and reproduction, and are eliminated through feces. How much energy that is available for growth and reproduction could be dependent on how much energy that is eliminated as feces. Since the naturally fed animals are exposed to *ad libitum* concentrations of food they are also likely to get smaller energy absorption because they will be spending more energy eliminating more feces than the food limited animals (Navarro & Winter, 1982). This could be one possible reason why the naturally fed animals have a significantly lower scope for growth than the food limited animals (Figure 3.4.1). Scope for growth can be defined as the difference between absorbed energy, often gained from food, and energy which is lost through energy-demanding metabolic processes (Sobral & Widdows, 1997). According to the energetics and scope for growth in our study, the food limited animals should have energy available for growth in all treatments, except for in the combined pCO_2 and temperature treatment. However, the actual growth results of these animals does not conform to that. Looking at the scope for growth, the food limited animals seems to tolerate elevated pCO_2 and elevated temperature separately, but when the animals are stressed by both pCO_2 and temperature they show a negative scope for growth.

4.4 Growth

In Exp. 1, the food limited animals had a significantly lower growth than the naturally fed animals. From these results it seems like the food limited animals are more prone to temperature changes than the fed animals (Figure 3.5.1), which implies that growth could be dependent on food availability (Auer et al., 2015), and is what was expected to see since the main process of absorbing energy, utilized for processes like growth, is through feeding (Navarro & Winter, 1982). The food limited animals were actually shrinking, showing a negative growth rate, which is consistent with their low MO_2 response reflecting their low energy demand (ATP) compared to the naturally fed.

Metabolic rate can be discussed as the standard metabolic rate (SMR) and maximum metabolic rate (MMR), which is equivalent to aerobic scope (AS). SMR is the minimum energy required to maintain and sustain life while MMR/AS is the excess energy available to be spent on other functions, i.e. growth. As mentioned under paragraph *4.1 metabolic rate*, food availability and quality can affect metabolic rate which can change the AS and hence the energy allocated to growth, among other functions. This is supported by Auer et al. (2015) who showed that AS and SMR had interactive effects on growth which depended on food availability. AS and SMR had no effect on growth at low food availability, resulting in no energy available for growth, unlike at *ad libitum* where AS had a positive effect on growth (Auer et al., 2015). This sounds plausible since the animals should have access to more energy at *ad libitum* which can potentially increase AS and lead to increased growth, which seems to conform to our growth results in both experiments where the fed animals has the overall highest growth. However, the access to energy is not only dependent on food availability, but also on CR and absorption efficiency. The food limited animals in Exp. 2 seems to have a high energy absorption and scope for growth, yet they are shrinking in size (growth rate) in all the different pCO_2 and temperature treatments. A possible explanation could be a plastic response of dwarfing to the stressful state of low food availability combined with and increasing temperature, which has been found as an adaptation to elevated pH in previous studies (Garilli et al., 2015). The 'Lilliput Effect' is a phenomenon where a reduction in size is seen among the biota after a mass extinction has occurred. This dwarfing in size is an adaptation through plasticity to the changed conditions following such an event (Harries & Knorr, 2009). The occurrence of such an adaptation has been looked into

and found in e.g. gastropods and polychaetes as a reaction to elevated pH. These reductions in size were seen although the energy absorption was higher in the elevated pH compared to the normal pH conditions (Calosi, Rastrick, et al., 2013; Garilli et al., 2015). The size reduction of the food limited animals might therefore be seen as a way to cope with the changing conditions although their energy absorption is generally higher than the fed animals.

As an organism grows the metabolic rate will seemingly increase as well since bigger animals should have the ability to consume more oxygen than smaller animals. However, smaller animals could use more oxygen per gram than larger animals because of their potentially larger surface-to-volume ratio. The relationship between metabolic rate (R) and body mass (M) is typically expressed as a power function, $R=aM^b$, where *a* is the intercept and *b* is the scaling exponent. The value of *b* has been debated for decades and the most supported values has been suggested at 0.67 (2/3) (White & Seymour, 2003) and 0.75 (3/4) (West et al., 1997). The value of this scaling exponent however, has in later studies been suggested to not be a universal value, but rather a value that will range between 0.5 and 1 depending on the species. This is supported by several studies on fish (Bokma, 2004; Glazier, 2005) and recently by Killen et al. (2010) where the scaling exponent was shown to be between around 0.7 in pelagic species and around 0.86 in bathyal species (Killen et al., 2010). By using this power function Urbina and Glover (2013) was able to describe the relationship between the body mass and oxygen consumption of their study fish. Under normoxic conditions the metabolic rate was found to scale linearly with body mass at a value less than isometry, meaning that the value of *b* would be less than 1 (Glazier, 2005). By using this scaling relationship it was calculated that fish of a smaller size had a higher metabolic rate than larger fish (Urbina & Glover, 2013). This could help explain the results in our study where the animals in Exp. 1 were generally of a smaller size with a mean mass of 0.81 g compared to the animals in Exp. 2 which had a mean mass of 2.6 g. This example can also compare to the results of Exp. 2 where the food limited animals had a generally high scope for growth, while they in reality were shrinking in size.

5.0 Conclusions

Comparing ocean warming (OW) and ocean acidification (OA), the ascidians in our study seems to be more sensitive to the combination of OW and OA than to the effects of OW alone. When the food availability is low the animals seem to compensate for this by lowering their MO_2 which is making them more resilient to the increasing temperatures. The food limited animals seem to have a higher thermal tolerance than the naturally fed animals. The animals' thermal window could give an idea of where they can be geographically distributed on a global scale with today's climate. With the future OW and OA, a shift in the animals geographic distribution may be prone to happen over time since the animals' thermal windows are narrow, which helps to keep the maintenance costs low (Portner & Farrell, 2008). These shifts in conditions will affect the growth response, which is depending on several factors like food availability, clearance rate, energy absorption and scope for growth. *C. intestinalis* seemed to have a lower toleration level of the combined effects of OW and OW, which is seen in their growth response. Interestingly, the food limited animals' scope for growth implied that they should be showing a positive growth response. However they were actually shrinking in size. When animals are subjected to stress from OW and OA the maintenance of homeostasis become more energetically expensive. Based on the results of our study, and others (Mackenzie et al., 2014; Mayor et al., 2015), *C. intestinalis* seems to compensate for this increasing energy demand by re-allocating energy away from energy demanding processes like growth and respiration in order to survive. This is particularly evident in our results when the animals are subjected to low food availability. It seems clear that the food availability is an important stressor *C. intestinalis* can be subjected to, which can have various effects when in combination with other stressors. However, there are still the need for more studies that includes multiple stressors as climate change occurs with multiple stressors concurrently (Doney et al., 2012). This is necessary in order to obtain a better understanding of the future acute and long-term effects of global climate change.

6.0 References

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