Green sea urchin (*Strongylocentrotus droebachiensis*, Müller) in aquaculture: the effects of environmental factors on gonad growth

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Preface and acknowledgments

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Last, but not the least, I would like to thank my family who has always supported me, and my dear Kari for her patience.

CONTENTS

1 Introduction

This presentation is based on the papers presented in the second part of the thesis. The thesis evaluates *Strongylocentrotus droebachiensis* (Müller), as a new species for aquaculture, in relation to environmental factors. In the thesis, I have evaluated some of the most important environmental factors that can effect gonad growth in adult green sea urchins. These include the effects of temperature, urchin body size, season, stocking densities and different water quality parameters (ammonium, nitrite, oxygen, carbon dioxide) on the survival, gonad growth, feed intake and feed conversion ratio of *S. droebachiensis*.

There is a scarcity of information regarding water quality parameters for urchin aquaculture and the available results are often only indicative. Given the steady increase in interest in sea urchin aquaculture of a range of urchin species around the world, there is a need for these parameters to be clearly defined.

This thesis discusses the results of the present study in relation to sea urchin aquaculture and also suggests some future directions for research.

2 The species

The invertebrate phylum Echinodermata is the largest group of marine deuterostomes (Yokata, 2002a). Echinoderms develop by indeterminate and radial cell cleavage, and develop body cavities by enterocoelic pouching (Smith, 2003). The body cavities are then modified in to a series of fluid-filled chambers that allow nutrition transfer (Smith, 2003). Four classes represent the major division: Stelleroidea (sea star), Echinoidea (sea urchin, sand dollar, heart urchins), Holothuroidea (sea cucumbers) and Crinoidea (sea lilies, feather stars) (Yokata, 2002a; Smith, 2003).

2.1 Geographic range

Strongylocentrotus droebachiensis, commonly known as the green sea urchin, is the most widely distributed member of the family Strongylocentrotidae (Stephens, 1972; Scheibling & Hatcher, 2001). *S. droebachiensis* has an arctic-boreal distribution (Sivertsen, 1997; Scheibling & Hatcher, 2001; Blicher et al., 2007). In the North Atlantic, it is found in Northern Europe and on the East Coast of the Canada and USA (Scheibling & Hatcher, 2001; Devin, 2002). In the eastern Pacific, it is found as far north as Point Barrow, Alaska, and southward to Washington State. In the North Pacific, it extends along the east coast of Siberia to the middle of the Kuril Island chain and the east coast of Sakhalin Island (Palumbi & Wilson, 1990; Scheibling & Hatcher, 2001; Andrew et al., 2002).

2.2 Biology

S. droebachiensis is a cold water, long-living, slow-growing species that is reported to have a life span of 45 years (Percy, 1972; 1973; Blicher et al., 2007). It is an osmoconformer with a limited capacity for osmoregulation (Lange, 1964; Schmidt-Nielsen, 1997). The green sea urchin exhibits great phenotypic plasticity of growth in response to environmental factors (Sivertsen, 1997; Russell, 1998; Blicher et al., 2007). *S. droebachiensis* ranges from 0 to 300 m in depth, but it is most commonly found in the shallow subtidal zone on rocky bottoms from 0 to 50 m (Jensen, 1974; Sivertsen, 1997; Scheibling & Hatcher, 2001). *S.*

droebachiensis is a benthic omnivore grazer, and it possesses a highly developed feeding apparatus known as Aristotle's lantern (Scheibling & Hatcher, 2001). It contains five extremely hard, pointed teeth which are adapted to scrape surfaces very efficiently. New tooth material is produced at a rate of about 1 mm to 1.5 mm per week (Barnes, 1987; Devin, 2002). Throughout the distribution range *S. droebachiensis* is commonly associated with Laminarian kelps. At high population densities, the sea urchins destructively graze kelp beds forming extensive barrens dominated by encrusting coralline red algae. They can persist in these barrens indefinitely, precluding the regrowth of erect macro algae (Christie et al., 1995; Sivertsen, 1997; Scheibling & Hatcher, 2001; Hagen, 2008). As an example, in the early 1970s the *S. droebachiensis* population along the Norwegian coast north of 63 °N started to increase, resulting in overgrazing so that in many areas the kelp forests were transformed to barren ground (Sivertsen, 1997). Despite the large populations of green sea urchin along the Norwegian coast, a commercial fishery has never become a success in Norway. A major reason is a very variable and generally low gonad content; with gonad content often well below 10% of the total weight, which is considered to be a minimum commercial standard (Sivertsen, 1997; James, 2007; Woods et al., 2008)

2.3 The edible sea urchin

About 1000 species of sea urchins have been identified (Yokota, 2002a). Only 16 species of sea urchins are harvested for food worldwide, with a total production of about 117,000 tonnes in 1995 (Keesing & Hall, 1998). By 2001, this had dropped to 80,000 tonnes due to over fishing, pollution and lack of proper fisheries management (Andrew et al., 2002). The major species taken are those in the three big producing countries: Chile (*Loxechinus albus*), Japan (*Strongylocentrotus intermedius*, *Strongylocentrotus nudus*), and United States of America (*Strongylocentrotus franciscanus*, *S. droebachiensis*) (Hagen, 1996; Keesing & Hall, 1998; Andrew et al., 2002; Agatsuma, 2007a,b.).

It is the gonads (more usually and loosely termed "roe"), which represent the edible part of the urchins (Figure 1). The product harvested for the fishery is the gonads of both sexes. Sea urchin gonads consist of two major cells types: (I) germinal cells, with undergo mitotic proliferation and meiotic reduction to produce large quantities of gametes, and (II) nutritive phagocytes (NP), which undergo cyclical depletion and renewal of macromolecules to provide the nutrients and energy required by the developing gametes (Holland & Holland, 1969; Kobayashi & Konaka, 1971; Lawrence, 2001; Wasson & Watts, 2007).

Figure 1: The green sea urchin *Strongylocentrotus droebachiensis*, dissected vertically in half and showing the yellow gonads, which is the edible part.

During the annual reproductive cycle, gonads of both sexes of *S. droebachiensis* pass through a characteristic series of structural changes (Walker et al., 1998b; Walker et al., 2001). These changes can be classified according to the activities of the two major populations of cells that compose the germinal epithelium. Following the phagocytosis of the relict gametes after spawning, gonad growth accelerates through the summer, first through the accumulation of nutritive phagocytes, reaching maximum rates in autumn when the proliferation of primary oocytes and initiation of vitellogenesis begins (Scheibling & Hatcher, 2001). The maturation and storage of ova and sperm proceeds through the winter as gonad mass continues to increase at a decelerating rate into early spring, when the spawning begins (Scheibling & Hatcher, 2001). Once gametogenenesis starts, stored nutrients, which consist mostly of major yolk protein in both females and males, are mobilized from the NP's to the germ cells, supporting gonial cell division and growth and/or differentiation (Walker et al., 2007). The linkage between these two processes, i.e. gametogenesis and nutrient utilisation, has an important role for aquaculture as it may be possible to delay gametogenesis, and maintain the quality of the gonads going to the market. The reproductive stage that produces gonads that is most preferred in international markets is the inter-gametogenesis and NP phagocytosis stage: this is when the ovaries and testis contain fewer gametes relative to somatic cells (NP) (Walker & Lesser, 1998a). In general, wild *S. droebachiensis* in good condition develop macroscopic gonads by the spring of their third year of life in nature (Raymond & Scheibling, 1987; Sivertsen, 1997).

The quantity and quality of the gonads in sea urchins is vital in the market and is considered critical to the profitability of the processing operation. High quality sea urchin gonads are characterised by large size, firm texture (containing few or no gametes), consistently high sensory scores, and yellow to orange colour (Yokota, 2002b; James, 2007; Woods et al., 2008). There is a long tradition of consuming sea urchin gonads in many cultures, particularly in Asia, Polynesia, the Mediterranean and Chile (Andrew et al., 2002). Modern commercial fisheries are focused on the Japanese market, which consumes more than 80 percent of the world's production (Hagen, 1996; Keesing & Hall, 1998; Andrew et al., 2002; Yokota, 2002b). Sea urchin gonads is a premium food in Japan, where it is eaten raw as sashimi, served with rice as sushi, or preserved in small bottles mixed in brine or alcohol and salt (Kramer et al., 1979; Yokota, 2002b). There are various methods for preservation of sea urchin roe, and the products can be consumed raw or processed (Yokota, 2002b). Snacks flavoured with sea urchin roe are also common (Yokota, 2002b).

2.4 Aquaculture of sea urchin

It is a general trend that most aquaculture operations of marine species do not start until the wild stocks fishery has been reduced to a point where the earnings and lifestyle of the participating fishermen are negatively affected (Robinson, 2003). Increasing demand for sea urchin gonads has in the last decade led to overfishing of natural populations (Keesing & Hall, 1998; Andrew et al., 2002). As overfishing continues to weaken world sea urchin populations, there has been an increased interest in sea urchin aquaculture as a means to supply future markets. Several possible approaches have been taken, such as reseeding natural habitats with farmed juveniles (Yokota, 2002b), gonad enhancement of adult sea urchins harvested from wild populations (Fernandez & Caltagirone, 1994; Klinger et al., 1997; Lawrence et al., 1997; Kelly et al., 1998; Robinson & Colborne, 1998; Olave et al., 2001; Pearce et al., 2002a,b,c.; Mortensen et al., 2003; James, 2006b), and finally land-based closed-system echiniculture allowing control of each phase of the animals biological cycle (Le Gall, 1990; Grosjean et al., 1998; Devin, 2002).

For the last two approaches, there is a strong need to establish feed types and feeding regimes that yield high quality gonad at acceptable costs. The use of formulated feeds is a common element in aquaculture for reasons of availability, consistency of quality and composition, water stability and ease of use. These factors are all crucial for the establishment of large-scale aquaculture of sea urchins (Caltagirone et al., 1992; Lawrence et al., 2001). Several formulated feeds are now available, among them the "Lawrence diet" patented in USA, a formula developed at St. Andrews Biological Station in New Brunswick (Dr. Shawn Robinson), a feed developed at Ross Island Salmon Ltd (Dr. Christopher Pearce), a moist feed developed by NIWA (based on a diet formulation developed at the Norwegian Institute of Fisheries and Aquaculture Research Ltd) in New Zealand (Dr. Phil James) and a dry extruded new feed developed by the Norwegian Institute of Fisheries and Aquaculture Research Ltd in Tromsø (NIFA- feed) (Woods et al., 2009). To date, most studies dealing with formulated feeds have examined the effects of these diets on gonad production (de Jong-Westman et al., 1995a,b; McBride et al., 1997; Pearce et al., 2002a,b., Pearce et al., 2003; James, 2007;Woods et al., 2008), nutrition and somatic growth (Klinger et al., 1998; Kennedy et al., 2007).

For a number of sea urchin species, gonad growth is shown to be faster with formulated feed than with natural food sources, such as algae (reviewed by Lawrence et al., 2001). Far less research has examined the effect of different feed formulas upon quality criteria other than gonad size, such as colour, taste and texture. However, recent studies have demonstrated that gonad colour (Goebel & Barker, 1998; McLaughlin & Kelly, 2001; Robinson et al., 2002; Watts et al., 1998), texture (Pearce et al., 2003) and taste (Pearce et al., 2003; McBride et al., 2004, Siikavuopio et al., 2007; Woods et al., 2008) can be manipulated through diet composition. In order to enable further development of formulated feeds and feeding regimes, it is necessary to try to identify the biochemical components associated with high quality gonad and furthermore how they are influenced by diet. Relatively fewer studies have been carried out on systems for on-growing sea urchins, either in land-based or sea-based systems (Grosjean, 1998; Le Gall, 1990; Devin, 2002; Aas, 2003; Robinson, 2003; Dagget et al., 2005; Pearce et al., 2005; James, 2006a,b; Kennedy & Robinson, 2007, James, 2007). There are also relatively few systematic studies aimed at understanding how the many environmental factors (e.g. temperature, nitrogen compounds in the water and dissolved gases in the water) influence gonad growth of sea urchins in aquaculture (Basuyaux & Mathieu, 1999; Pearce et al., 2005, James et al., 2007, James, 2007; James et al., 2009).

3 Aim of the thesis

The main objective of the work reported here is to increase the knowledge of some of the most important biotic and abiotic factors affecting gonad growth of adult green sea urchin, *S. droebachiensis,* as a new promising candidate species for aquaculture. Most aquaculture operations aim to maximise the growth rates of cultured animals. Since only the gonads of urchins are eaten, there are two different stages of growth that need to be addressed. Firstly, the somatic growth of juvenile individuals should be maximised until the urchins reach sexual maturity and a reasonable market size. Secondly, after sexual maturation, the urchins should be stimulated to put as much as possible of their energy into nutritive cell development in order to maximise gonad yield. The focus in this thesis is on some of the most important environmental factors affecting gonad growth of adult urchins. Experimental studies have been carried out in the laboratory and the capacity of the adult urchins to survive, feed, and produce gonads under aquaculture conditions has been related to studies of:

1. Optimal temperature for gonad growth related to season and body size
2. Effects of animal handling during harvest and transport on survival and gonad growth
3. Effects of nitrogen waste compounds on survival and gonad growth
4. Effects of dissolved gases in the water on survival and gonad growth
5. Effects of optimal stocking density for gonad enhancement in two different holding systems

The results are discussed in relation to sea urchin aquaculture and are used to develop a set of criteria that should be considered for on-growing adult *S. droebachiensis* in aquaculture. These results can be used to calculate necessary water renewal rates, maximum animal densities, and give recommendations on optimal temperature and water quality requirement in relation to adult *S. droebachiensis* held in captivity.

4 Methods and materials

This thesis presents results from controlled experiments with *S. droebachiensis.* All of the experiments were carried out at Tromsø Aquaculture Research Station. The experimental animals were sampled in the Kvalsund between Ringvasøya and Kvaløya close to the Research Station in Tromsø by experienced scientific divers. The experimental animals were all adults.

Feed

In all experimental setups, the sea urchins were fed the same moist NIFA feed as described by Mortensen et al. (2003) (Table 1).

Table 1: List of ingredients (% wet weight) and proximate composition of the formulated NIFAfeeds (% dry weight basis)

Ingredients used in diet	%
Fish skin (*Pollachius virens*)	89.05
Kelpmeal (*Laminaria hyperborea*)	8.90
Starch (potato meal)	1.78
Glucose	0.09
β-carotene	0.02
Ascorbic acid	0.02
Transglutaminase	0.13
Composition	
Protein	59.1
Lipid	0.5
Carbohydrate*	24.6
Ash	15.7
Moisture	62.6

(* determined by difference)

The use of fish skin, in combination with transglutaminase, to enhance the gel binding features, gives a sea urchin feed with a high stability in sea water. Stability tests have revealed that the formulated feed can endure immersion in sea water for seven days at a temperature of 14 ºC without a significant reduction in consistency, making it appropriate to administer at a frequency of once a week. This relatively slow feeding frequency is not optimal in relation to optimising the feed quality. But in relation to the sea urchins' feeding behaviour, it is possibly the best way to feed the adult sea urchins (the sea urchins are attached to the feed until it is completely consumed). Feeding once per week also reduces negative interaction (e.g. collisions) between the urchins as they search for feed (see paper IX).

Feeding procedures

In all of the experiments, the urchins were fed in excess. Because of the stability of the feed in sea water, ingested feed could be calculated as the difference in dry weight mass between feed provided and feed remaining after the feeding period (usually one week).

Experimental setup

Most of the experiments (papers I, II, III, IV and X) were carried out in raceways (400 cm long x 42 cm wide x 15 cm deep). The raceways were equipped with perforated false bottoms which allowed faeces, but not feed particles, to fall through and be flushed out. Raceways were divided into compartments (12 cm long x 10 cm wide x 15 cm deep) for single animals. Each raceway was supplied with filtered seawater, spread by means of perforated pipes above the water surface (Fig 2a). The experiments in papers V, VI, VII and VIII were carried out in circular fibreglass tanks (from 30 l to 100 l). Paper IX was carried out in 755 l plastic tanks; length: 155 cm, width: 108 cm, height: 69 cm (Sæplast double-walled polyethylene container). The tanks were compartmentalised into eight chambers by vertical lamellae (length: 60 cm, width: 15 cm, height: 40 cm) made of fibreglass (Fig. 2b). The sea urchins were unable to pass from one chamber to another. The tanks were equipped with a perforated false bottom with a drain underneath. The drain facilitates a quick removal of faecal material and debris of excess food. In addition, the tanks were drained completely and cleaned once a week. Water temperature and oxygen saturation was measured daily or weekly.

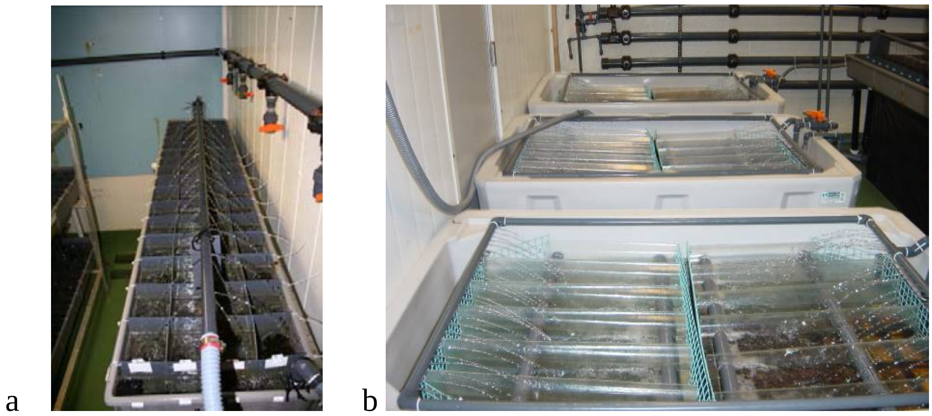

Figure 2: The experimental setup with raceways divided into compartments (a) and tanks with vertical lamellae (b)

The sea urchins were subjected to a simulated natural light regime (~70˚N) and a maximum light intensity of about 150 Lux at the water surface. Raceways were cleaned once a week by flushing the space beneath the false bottom.

In papers I, II, III and IV, urchins were held individually in square formed chambers (Figure 2a). In the paper V, VI, VII, IX and X, the sea urchins were held in groups. The duration of all experiments (except the long-term experiment in paper III) was approximately eight weeks, which is the usual period needed to increase the gonad size of wild caught *S. droebachiensis* to marked size using the NIFA feed (Mortensen et al., 2003).

Basic calculations and acronyms

Feed intake (FI)

In all experiments daily feed intake (FI) was calculated as FI = g feed offered – g residual feed /days. Feed intake is commonly given as grams of feed per day per animal in the sea urchin literature (Lares & McClintock, 1991; Scheibling & Hatcher, 2007).

Feed conversion ratio (FCR)

Feed conversion ratio (FCR) with respect to gonad growth denotes the amount of feed (g) necessary to increase the gonad weight by one gram. The use of FCR in relation to gonad growth is not so commonly used in the sea urchin literature, mainly because of little focus on aquaculture in earlier works related to sea urchins. There are no methods available to measure gonad growth *in vivo*. Therefore, in all experiments gonad growth has been calculated from the gonad content of random samples of urchins taken at the start and for all animals at the end of the different treatments.

Gonad indices (GI)

Gonad indices are indicators of gonad development that are independent of urchin size (James, 2007). Various authors have indicated the size of the gonad using various gonad indices (Mottet, 1976). The indices express the size of the gonads relative to the size of the whole urchins. The most commonly used indices is the gonad index (GI = gonad wet weight (g)/whole body wet weight (g) * 100). This definition is used in the papers in this thesis.

5 Results and Discussions

5.1 Temperature

Background

Gonad yield is an important factor in the commercial exploitation of sea urchins (James, 2007). For gonad enhancement of a wild caught adult, it is necessary to determine the water temperature that results in highest gonad yield. In general, little systematic work has been done on optimum water temperature ranges for different species of sea urchins in respect to gonad growth (McBride, 1997; Spirlet, et al., 2000; Shipigel, et al., 2004; Pearce et al., 2005, James et al., 2009). For aquaculture to be carried out efficiently and profitably, it is important that the effects of water temperature on feed intake, feed conversion and growth rates of the cultured species are known and not only the tolerance limits to the animal. Water temperature is one of the most important factors governing metabolic processes in poikilothermic animals such as sea urchins (McBride et al., 1997; Spirelt et al., 1998; Brockington & Clarke, 2001; Shipigel et al., 2004, Pearce et al., 2005). Temperature is known to influence both ingestion and metabolism, and will affect growth (Jobling, 1994, Paper I). When sea urchins experience temperatures outside the normal range, the effects may be negative. Environmental disturbances will interfere with a range of biochemical and physiological systems essential for the sustenance of life. Exactly what constitutes the normal environmental range will vary from species to species and depend upon the evolutionary history of the species. For example, *S. droebachiensis* is a cold water species that is tolerant to low temperatures and dies at temperatures considered normal for *S. nudus* (Agtusuma, 2007b; Scheibling & Hatcher, 2007).

Temperature influences the distribution and abundance of *S. droebachiensis*. Adult *S. droebachiensis* are well adapted to low water temperatures (-1.9 ºC) and the upper temperature tolerance limit is about 22 ºC (Percy, 1972; 1973;1974; Pearce et al., 2005; Blicher et al., 2007). For adult green sea urchin, *S. droebachiensis* no systematic research has been done on the influences of different temperature on feed intake, feed conversion ratio and gonad growth (Pearce et al., 2005; Scheibling & Hatcher, 2007). Determination of an optimum thermal regime for gonad growth is pertinent as this species can exist at a large range of temperatures. The next chapter will address some of the most important results from papers I, II and III on the effects of different temperature in relation to sea urchins size, season on gonad index, feed intake, feed conversion ratio and metabolism of adult *S. droebachiensis.*

5.1.1 Effects of temperature on gonad growth in relation to body size

In paper I and II, one off the overall objectives were to investigate the effects of different temperature regimes on gonad growth of adult green sea urchin *S. droebachiensis*. Figure 3 (Paper I) illustrates the effects of temperature on gonad growth of three different size groups of adult *S. droebachiensis:* 40 g (S), 65g (M), 100 g (L).

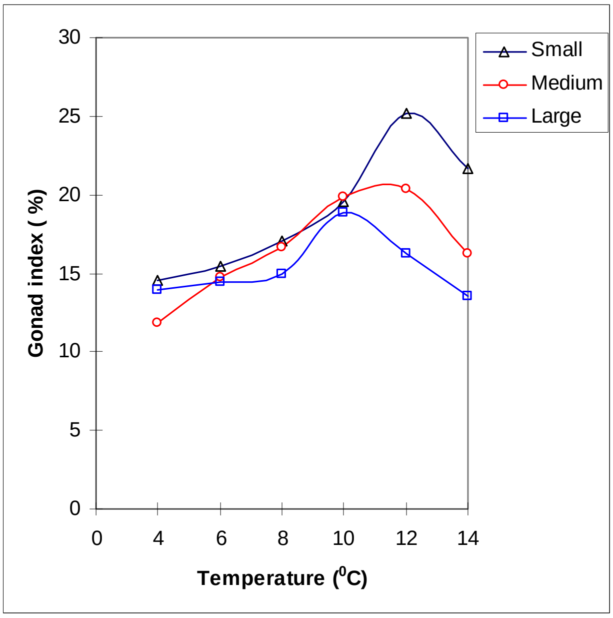

Figure 3: Effects of temperature on gonad index of three size groups of adult *S. droebachiensis* (40 g (S), 65g (M), 100 g (L) (paper I)

Paper I (Fig. 3) and II (Fig.4) illustrate that GI increases with temperature up to a peak and thereafter decreases depending on body size and season. Size-related differences in increase in GI were observed (paper I), where the smallest urchins showed greater percentage increase in

GI followed by medium and large size groups. Pearce et al. (2004) working with the same size classes of *S. drobachiensis*, also found that increases in GI were greatest in the smallest size group with gonad growth being inversely related to body size. Pearce et al. (2004) postulated four possible reasons for size-related differences in urchin gonad increase when enhanced: 1) larger urchins have proportionately less energy available for gonad production due to increased basal metabolic and/or maintenance costs, 2) larger urchins may have lower feed consumption rates, 3) larger urchins may have lower absorption or assimilation efficiencies, and/or 4) larger urchins may require proportionately more energy for equivalent increase in percent gonad yield. In the current experiments (paper II and III), there is evidence that large urchins have the lowest feed consumption rate (in relation to body weight), and there is a feed intake-body size relationship according to Pearce et al. (2004). In paper III, there was evidence of lower assimilation efficiencies in relation to size at the same temperature, which supports the proposal that larger urchins have lower absorption or assimilation efficiencies.

5.1.2 Effects of temperature on gonad growth in relation to season

Figure 4 illustrates the effects of temperature on gonad growth in relation to different seasons (summer/autumn and winter) of adult *S. droebachiensis* (mean weight, 65 g) described in paper II.

The gonad growth is strongly linked to temperature (papers I and II) and season (paper II), where GI is increasing with water temperature and peaks at 10 °C in summer and at lower temperature in winter (8 °C). An interesting aspect of these results is that the *S. droebachiensis* under winter conditions has almost the same gonad index at temperatures up to 8 °C, compared with sea urchins under summer/autumn conditions (Fig. 4). A simple explanation may be that temperatures above 8 °C in winter are suboptimal for adult *S. droebachiensis*. Stephens (1972) found that larvae of *S. droebachiensis* developed normally at a temperature up to 10 ºC, but observed gross asynchrony in embryonic development above 10 ºC and irreversible arrest of cell division above 12 ºC. Based on these results, he suggested that recruitment of larva to adult population is restricted to areas where temperatures in winter/spring do not exceed 10 ºC. The results in paper II can, therefore, possibly be linked up to this environment adoption of adult *S. droebachiensis*. To fully understand these results it would be ideal to include a histological study of the gonads to identify whether changes in the reproductive stage of the urchins account for these results (James, 2007). Possible size dependent seasonal variation in optimal temperature should also be investigated.

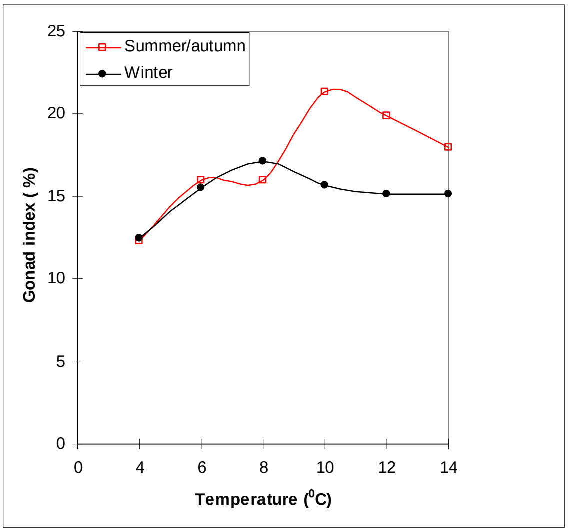

Figure 4: Effects of temperature on gonad index in relation to season: summer/autumn and winter of adult *S. droebachiensis* (65g (M)) (Paper II)

The results in paper II suggest that optimal temperature regimes will differ according to season and the best combination for gonad growth of adult *S. droebachiensis* in size class M would be 10-12 °C in summer and 6-8 °C in winter. Use of this recommendation in relation to production of juvenile *S. droebachiensis* become known from the works to Pearce et al. (2005) where they suggest that juvenile *S. droebachiensis* should be reared at 9-13 °C in order to optimise production for aquaculture.

5.1.3 Effects of temperature on feed intake and feed conversion ratio

For potential sea urchin farmers it is necessary to determine the feed intake and feed conversion ratio to minimise the feed cost. Overfeeding can also lead to suboptimal water quality that can have negative effects on gonad growth and survival rate of green sea urchins, as illustrated in papers V, VI, VII and VIII. In both papers I and II, the feed intake increased significantly with temperature in a linear manner. In paper II, size-related differences in feed intake were observed in *S. droebeachiensis*, with smaller urchins having a greater percentage increase in feed intake (according to kg of sea urchin) at the same temperature, compared to the medium and largest size urchins.

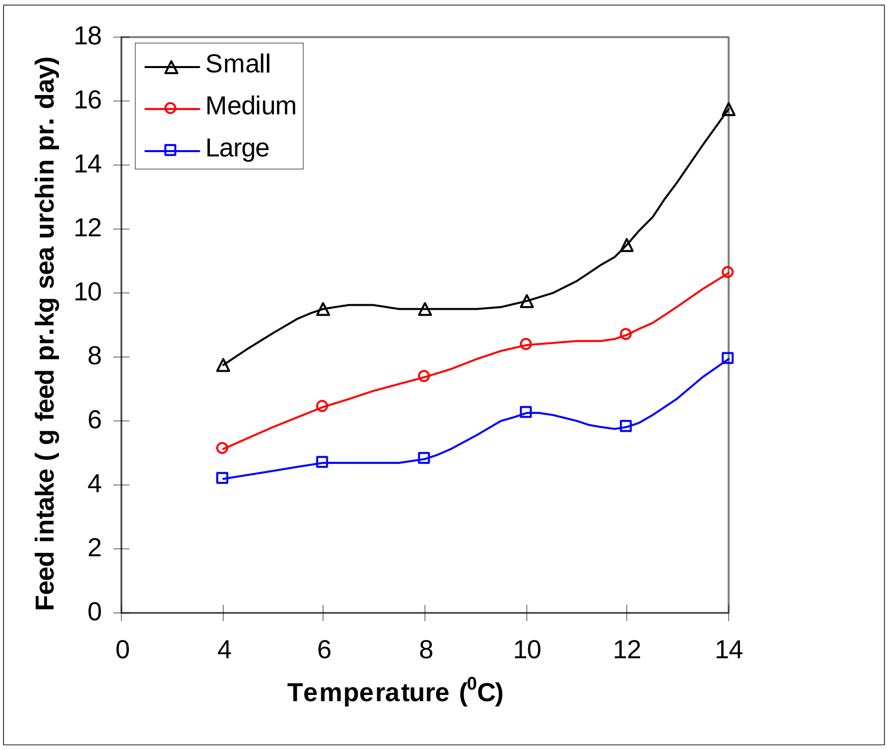

Figure 5: Effects of temperature on FI (g feed /kg sea urchin/day) of different size groups of adult *S. droebachiensis* (40 g (S), 65g (M), 100 g (L) in relation to different temperature (Paper I)

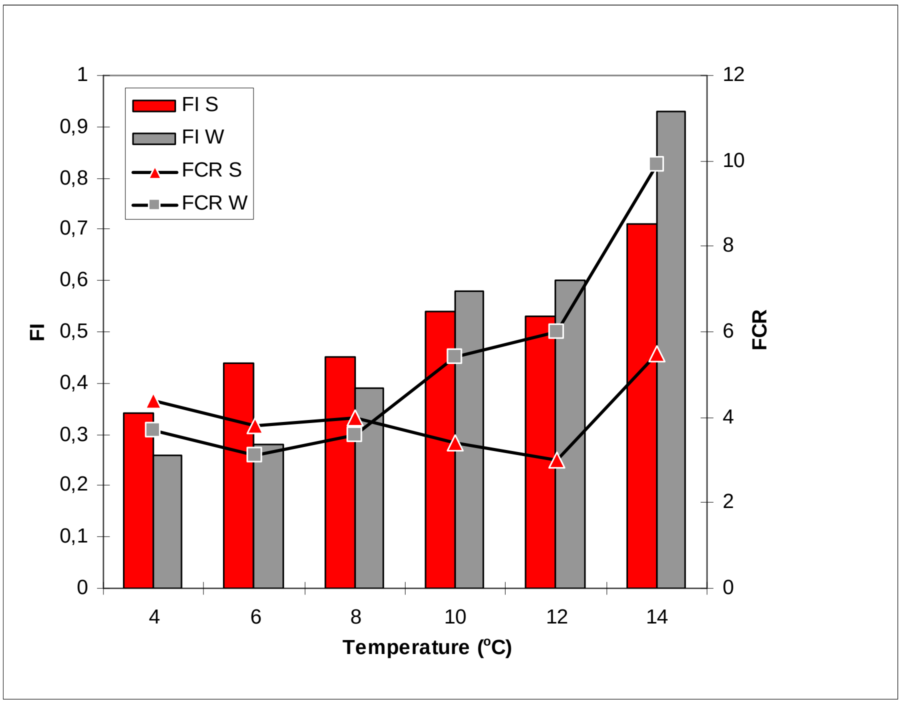

Figure 6: Effects of temperature on FI (g feed per sea urchin per day) and FCR (g feed per g gonad increase) of medium size *S. droebachiensis* in relation to season (S= summer/autumn, W= winter) (Paper II).

In paper II, the animals in the medium size class were tested at the same temperature regimes in summer/autumn (July - September) and under winter conditions (November - January). In summer/autumn the sea urchins held at 12°C had a better FCR compared to animals held at 8°C. The situation was reversed in winter, where sea urchins displayed better FCR at 8°C than sea urchins held at 12°C (Fig. 6). Paper II illustrates that FCR varied significantly between temperature treatments and season. One explanation may be that *S. droebachiensis* is possibly adapted to lower temperatures in winter compared to summer, regulated by endogenous cellular mechanisms. Possible biological clocks that track photoperiod allow organisms to predicate the coming season and adjust their physiology accordingly. Control of such clocks is highly relevant in determining the onset of gametogenesis in sea urchins is directly related to when urchin echinopluteus larvae will emerge in the plankton and begin feeding in spring.

Experimental setup with eggs and larvae from *S. droebachiensis* has illustrated that they are intolerant to temperatures above 10°C (Stephens, 1972; Motte, 1976; Himmelman, 1978). The winter experiment is near the spawning season for the *S. droebachiensis* and temperatures over 10 °C maybe too high for the adult *S. droebachiensis.* Overall, these findings in paper II suggest that optimum FCR of adult green sea urchins in size class M is achieved at higher temperatures in summer/autumn than in winter. This result illustrates possible risk of poor water quality due to the high FCR at the highest temperature (14°C) under either summer or winter conditions.

5.1.4 Feed intake in relation to short and long-term situation at constant temperature

The objective in paper I and II was to investigate the effects of temperature and season on gonad growth of sea urchins in relatively poor nutritional status with a GI under 10 percent. Paper III was a long-term experiment (848 days) where sea urchins were fed in excess and held at a constant water temperature near the optimum gonad growth temperature for adult *S. droebachiensis* (10 °C) according to papers I and II. These results show that FI of the adult green sea urchins is also strongly linked to the season at a constant temperature. Scheibling and Hatcher (2007) concluded in a summary of the ecology of the green sea urchin that feeding rates are not linearly related to temperature but generally show a strong relationship with the reproductive cycle, which itself varies seasonally with temperature. This conclusion appears to be correct in relation to a long-term study (paper III).

Most studies indicate that echinoderms have little ability to acclimate their neuromuscular systems to changes in temperature, so that organism activity including feeding is directly related to temperature (Lawrence, 1987). This appears to be correct in short-term experiments, (months) (papers I and II), but in relation to long-term studies (paper III) feed intake has a seasonal pattern that is likely to be linked to basic biorhythm. Basic biorhythm research describes that environmental cues, like photoperiod, serve to synchronise annual endogenous rhythms and, as such, the changes in photoperiod are likely to adjust the endogenous rhythms controlling seasonal events (Gwinner, 1981).

Circadian rhythms are not well known in echinoids although photoperiodic activity has been recorded for reproductive behaviours (Coppard & Campbell, 2005; Burke et al., 2006). Thus, the seasonality in feed intake observed in our study may, to a certain extent, reflect the state of reproduction rather than the photoperiod *per se.* Previous studies on sea urchins have indicated that the feed intake increases when the gonads are in a spent, recovering, and growing stage (McBride et al., 1997; Spirlet et al., 2000; McBride et al., 2004). However, the underlying links between feed intake and the reproductive cycle still remain unclear. According to Fuji (1967), *S. intermedius* exhibits an inverse relationship between feed intake and gonad index. Hence, as the body cavity becomes filled with gonad tissue, a simple reduction in the coelomic space, and probably also in the volume of the intestinal tract, would hamper the ingestion of feed (Fuji, 1967). This purely mechanistic hypothesis fits well with the results in paper III on the *S. droebachiensis*. In December 2001 at the end of the experiment, the GI values (33-35 %) had reached the highest level reported for this species (de Jong-Westmann et al., 1995a; Meidel & Scheibling, 1999), when the corresponding FI values were at their lowest.

From the literature it is known that green sea urchin is strongly linked to the kelp forest that has a seasonal growth pattern and seasonal variation in nutrition quality (Sivertsen, 1997). It is, therefore, possible that there is a link between nutrient access and seasonal variation in feed intake of *S. droebachiensis*.

In paper III, only one combination of light and temperature regime was used. There is information from studies of *S. droebachiensis*, that temperature in combination with different light regimes also influences the gametogenic cycle (Walker & Lesser, 1997; Garrido & Barber, 2001) and possibly thereby the pattern of seasonal rhythm of feed intake described in paper III.

Overall paper III indicates strong seasonal variations in feed intake. These results must be considered when feed tables are developed for sea urchin farmers undertaking gonad enhancement. If this is not taken in consideration, the resulting poor water quality increases the risk of mortality or low gonad growth rates (papers V to VII).

5.1.5 Effects of temperature on metabolism in relation to body size and feed

Knowledge of the effects of body weight, temperature and feeding on the metabolism of commercially valuable animals is important for the estimation of the energy and water requirement of the organism. This information is critical for the establishment of optimal culture conditions and management strategies. Respiration by sea urchins includes the uptake of oxygen from the environment at sites of gas exchange, the use of oxygen at mitochondria within the individual cells, and the excretion of waste gas to the environment (Steen, 1965; Schreck & Moyle, 1990). Respiration provides oxygen for aerobic conversion of energy contained in food to high-energy chemical bonds, such as those formed when adenosine diphosphate is changed to adenosine triphosphate (Schreck & Moyle, 1990). Oxygen consumption rate has become the conventional metabolic measure for invertebrates. Aerobic metabolism can be categorised as standard, resting routine, routine, and active (Schreck & Moyle, 1990). Paper I attempts to describe the oxygen consumption of the adult green sea urchin, *S. droebachiensis* in relation to body weight, temperature, resting and feeding states.

In general, temperature changes have considerable effects on many physiological processes (Jobling, 1994), e.g. the effects on the rate of oxygen consumption, which is a convenient expression of the overall metabolic activity of animal (Schmidt-Nielsen, 1997). Normally, a rise of temperature by 10 °C causes the rate of oxygen consumption to increase about twofold to threefold (Schmidt-Nielsen, 1997). The increase in rate caused by a 10 °C increase in temperature is called the Q_{10}. The low Q_{10} values for oxygen consumption among starved sea urchins and high Q_{10} values for feeding animals found in paper I make the green sea urchin well adapted to variations in feed supply. In cases when feed is absent, the respiratory energy loss is kept at a minimum irrespective of water temperature. These results are supported by results from different ecological studies with *S. droebachiensis* where it can survive for extended periods when food is unavailable (Himmelman & Steele, 1971). On the other hand, when feed is abundant at favourable temperatures, they may allocate their metabolism towards growth. This opportunistic feature is more pronounced for larger sea urchins. In paper I, the difference in oxygen consumption between fed and starved animals increased rapidly with temperature for all size classes (paper II). We did not examine the body composition of starved and fed animals and can, therefore, not exclude that growth of the digestive tissues may have contributed to the observed differences. However, the contribution is probably insignificant since the gut comprises only a smaller proportion of the total body weight (2-4

%) and does not undergo large seasonal changes compared to gonad tissues (Bookbinder & Schick, 1986). For reasons to be discussed below it is also unlikely that the increased oxygen consumption of fed sea urchins can be explained in terms of gonad growth. Hence, the higher oxygen consumption of fed animals is likely to be associated mainly with specific dynamic action, and may be due to increases in whole-animal protein synthesis (Weatherley & Gill, 1987).

Sea urchins lack an efficient system for transportation of oxygen from the environment to the internal tissues (Steen, 1965; Giese et al., 1966; Johansen & Vadas, 1967), and the gonads rely to a large extent on anaerobic metabolism. In a study on green sea urchins, Bookbinder and Shick (1986) estimated that 76 - 92 % of the substrate used for gonad metabolism was metabolised anaerobic. Accordingly, Giese et al. (1966) and McPherson (1968) found that the weight specific oxygen consumption of sea urchins was largely independent of changes in GI. The same lack of correlation is evident in paper I, since the oxygen consumption continued to increase with increasing temperature above the temperature where the gonad growth peaked (10-12 ºC). It is difficult to explain why both feed intake and oxygen consumption should increase at temperatures above 10 – 12 ºC while energy consuming processes like gonad growth, somatic growth and locomotory activity do not. However, a simple explanation may be that the regulatory mechanism securing a balance between energy intake and expenditure does not function adequately under these conditions, and that the animals are wasting energy by ingesting and processing feed in excess. Papers I and II show that the regulation of energy intake, in relation to energy expenditure, seems to be poorly developed, leading to a disproportionately high FCR when animals are fed at temperatures above 10 ºC. Papers I and II illustrate that more research needs to be carried out in relation to understanding mechanisms regulating feed intake and the feed conversion in adult green sea urchins.

5.2 Physiological tolerance limits and water quality requirements

Background

The profitability in gonad enhancement operations will depend on several factors, both biological and operational. For obvious reasons, gonad yield and mortality are the critical biological parameters regarding financial returns in gonad enhancement operations. In order for these operations to be successful, the sea urchins have to be kept under optimum conditions of feeding strategy, stocking density, and water quality, and the tolerance limits and optimum levels need to be established (Motnikar et al., 1998). The immediate objects in papers IV, V, VII, VIII, IX and X was to determine some of the most important environmental conditions that limit gonad growth.

There has been little focus in the literature on biotic and abiotic factors causing mortalities of adult green sea urchin in relation to aquaculture. There is some information from wild populations and experimental studies on low surface salinity killing small urchins during tidal fluctuation at shallow depths (Himmelman et al., 1984; Kashenko, 2007). There is also evidence that accumulation of drift algae creates hypoxic condition and causes mass mortality of *S. droebachiensis* (Dumont & Himmelman, 2004). In the literature sea-urchin embryo - larval has been used in predication and assessment of toxicity of marine pollution (Bellas, 2008; Rosen et al., 2008). Only a few studies have focused on the tolerance limits of adult sea urchins related to water quality and handling and transport stress (Basuyaux & Mathieu, 1999, James, 2007). In recent years, sea urchin larvae has been used as a model spices for understanding the effect of ocean acidification due to the calcification effects (O`Donnell et al., 2009).

Paper IV focuses on the transport and handling time of wild caught sea urchins in relation to possible effects on increasing mortality, GI, FI and FCR. In papers V to VIII, the most important water parameter has been studied according to survival, GI, FI and FCR.

5.2.1 Effects of handling on survival, feed intake and gonad growth

Commercial gonad enhancement operation in Norway will primarily rely on sea urchins harvested from the wild. As sea urchins are strongly attached to surfaces, removing them by hand or using tools can cause tissue damage, stress and eventually an increase in mortality. The transport methods and treatment during transport can also reduce the survival rate. Previous studies have reported a pattern of high mortality in the beginning of an experiment followed by a period of low or no mortality (Minor & Scheibling, 1997, James, 2007, Paper X). The aim of the study in paper IV was to describe the effects of handling and air exposure during harvest and transport on mortality and gonad growth of *S. droebachiensis* in a subsequent gonad enhancement trial.

Two experimental factors - handling (gentle and rough) and degree of air exposure (wet and dry) - were combined to form four different treatments: gentle/dry (GD), rough/dry (RD), gentle/wet (GW) and rough/wet (RW). In the subsequent roe enhancement trial, the highest mortality (exceeding 25%) was observed in the GD treatment. Mortality was 1.5% in RW and RD treatments, while no mortality was observed in the GW treatment. Mortality only occurred during the first four weeks after harvest. Desiccation appears to be the main cause of mortality. The low mortality in other treatments points to air exposure rather than mechanical stress as the main cause of mortality. There was a significant increase in gonad index for all the treatments during the gonad enhancement trial. However, the increase in gonad index was significantly lower in the RD compared to the other treatment groups. The lower gonad growth in RD was probably due to the high frequency of individuals with visual injuries, a frequency that was an order of magnitude higher than in the other treatments.

Overall, there was a clear relationship between visual injuries and gonad index, where individuals with injuries had a significantly lower gonad index at the end of the experiment compared to individuals without visual injuries. These results are supported by results from paper X where individuals with visual injuries had a significantly lower GI compared with intact sea urchins. The lower feed consumption and higher feed conversion factor observed in RD indicate that individuals with injuries have a reduced gonad growth due to a combination of reduced appetite (feed intake) and lower feed conversion efficiency. Regenerative potential is expressed maximally in echinoderms (Hyman, 1955), and regeneration involves both external and internal organs. Initiation of spine regeneration following damage leads to changes in resource allocation (Ebert, 1967, 1968, Edwards & Ebert, 1991). The effects of injuries on gonad growth are also supported by the results from paper IX, where sea urchins with injuries had a significantly lower gonad growth compared to sea urchin without injuries. According to Edwards & Ebert (1991), animals with spine damage appeared to allocate greater amounts of resources to regenerate the test and the spikes. It is, therefore, possible that the lower gonad growth in individuals with visual injuries observed in all these studies is caused by fewer resources being allocated to gonad growth.

The removal of sea urchins from tanks for servicing, density adjustments, size selection, cleaning, marketing etc., is also a necessary, intermittent husbandry activity. This handling can also cause tissues damage, stress and eventually an increase in mortality. One possible handling method to reduce tissue damage, stress and eventual increase in mortality is illustrated in an experiment with James (2007), where sea urchins were removed from the holding system for the water three times per week, but not removed from the holding unit. This had no impact on gonad growth or mortality.

It is, therefore, very important when new operating systems for sea urchins are designed and constructed that this information is considered.

5.2.2 Effects of nitrite and un-ionised ammonia on gonad growth, feed intake and survival

Background

Water quality in rearing systems is altered directly by the animals through their consumption of oxygen and their excretion of carbon dioxide, faecal matter and nitrogen wastes. In all aquaculture operations, especially those where the water is treated, it is desirable to know the maximum and minimum acceptable changes that may occur in water chemistry so that effort is not spent trying to achieve unnecessary water chemistry parameters.

In intensive closed or semi-closed rearing systems, even with frequent water changes, it is common to observe an accumulation of inorganic nitrogen, such as ammonia, nitrite and nitrate (Chen et al., 1989,1990; Grosjean, et al., 1998). High levels of nitrite and ammonia in the water are potential factors triggering stress and can cause high mortality in aquatic organisms (U.S. EPA, 1989; 1999; Harris et al., 1998; Basuyaux & Mathieu, 1999; Ip et al., 2001; Randall & Tsui, 2002; Timmons & Ebeling, 2007). Detailed knowledge of the impact of water quality factors on fundamental production characteristics such as growth performance, feed conversion efficiency and animal welfare is, therefore, needed in order to effectively exploit such systems.

Nitrogen occurs in aquatic environments in several forms: dissolved gaseous N_2 (from the atmosphere), dissolved gaseous NH_3 (most as waste from animal metabolism), ammonium ions (NH_4^+), nitrite ions (NO_2^-) and nitrate ions (NO_3^-) (Timmons et al., 2001). The term "ammonia" is often used to include both the dissolved unionised gas (NH_3) and the ionised NH_4^+, and as such is better expressed as total ammonia (TAN). The relative concentration of ammonia (NH_4^+ and NH_3) is a function of water pH, salinity and temperature. The un-ionized ammonia (NH_3) is the most toxic form of ammonia because of its ability to move across cell membranes (Timmons & Ebeling, 2007). An increase in temperature, pH or salinity increases the proportion of the un-ionized ammonia (Timmons et al., 2001).

The first and possibly only long-term experiment on the effects of ammonia, nitrite and nitrate on somatic growth of juvenile sea urchins, *P. lividus* was undertaken by Basuyaux & Mathieu (1999). Ammonia and nitrite is the principal end product excreted by the sea urchin, *P. Lividus* (Arafa et al., 2006). Nitrite is formed from ammonia and may accumulate in aquatic systems as a result of imbalances of nitrifying bacterial activity (*Nitrosomas sp.* and *Nitrobacter sp.)* (Colt & Armstrong, 1981; Wickins & Lee, 2002; Timmons & Ebeling, 2007). We do not know of any published studies on the effects of ammonia, nitrite and nitrate in relation to gonad growth of adult green sea urchin and papers V-VII are probably the first studies where the main focus has been on the effects of different water quality on gonad growth of adult green sea urchins.

The nitrite paper (VI) was designed to investigate the effects of chronic exposure to sub-lethal levels of nitrite, ranging from 0.01 to 10 mg N-NO_2 l^{-1}, on gonad growth and feed intake in adult green sea urchins. The levels of nitrite selected in paper V are based on the results from an experiment with the juvenile sea urchin *P. lividus* (Basuyaux & Mathieu, 1999), where the safe limits for *P. lividus* were found to be in the range of 1-2 mg N- NO_2 l^{-1} for somatic growth. In the present paper VI, gonad growth was significantly reduced by increased nitrite concentrations, with reduced gonad index already being apparent at 0.5 mg N-NO_2 l^{-1}. This result is lower than the safe limit for *P. lividus* which was found to be in the range of 1-2 mg N- NO_2 l^{-1} for somatic growth (Basuyaux & Mathieu, 1999). Authors have suggested that nitrite acts by oxidising haemoglobin into methaemoglobin, which has a characteristic brown colour (Daniel et al., 1987; Timmons & Ebeling, 2007), but a direct influence on haemoglobin is unlikely due to the sea urchins' lack of respiratory pigments (Basuyaux & Mathieu, 1999).

Since echinoids are osmocomformers, internal fluids are similar in their ionic osmotic concentration to the outside environment (Schmidt-Nielsen, 1997). Thus, body tissues within the coleomic fluid will then be subjected to accumulation of inorganic nitrogen that can have a negative impact and stress the animals, illustrated by poor FCR or high mortality in papers V and VI.

The levels of un-ionised ammonia selected in paper V were also based on the results from the experiment with the sea urchin *P. lividus* (Basuyaux & Mathieu, 1999). In paper V, the effects of chronic un-ionised ammonia (UIA) exposure on the green sea urchin, *S. droebachiensis,* were examined. Sea urchins were exposed to four concentrations of UIA [0.0001 (control), 0.015 (low), 0.030 (medium) and 0.070 (high) mg l^{-1}]. Sea urchin mortality was influenced by UIA concentrations and increased with increasing levels of UIA. The mortality in the high-concentration group increased throughout the experimental period and ultimately only 24% of the sea urchins survived. Compared to the control, gonad growth was significantly reduced at UIA concentrations above 0.015 mg N-NH_3 l^{-1}. In a previous experiment, Basuyaux & Mathieu (1999) did not observe mortality in sea urchins *P. lividus* held at the same test solution selected in paper V. However, this experiment lasted for only 15 days. UIA exposure is more likely to show its effects in long-term experiments and in the present paper, a pronounced mortality of adult urchin was observed both at medium and high UIA levels in the period following 15 days of exposure. Basuyaux & Mathieu (1999) found there was a significant negative influence on the somatic growth of *P. lividus* from increasing concentrations of ammonia (Basuyaux & Mathieu, 1999). Growth decreased by about 50 percent at 0.5 mg N-NH_3 l^{-1}.

No significant differences in feed intake were observed between unionised ammonia groups selected in paper V or at the different nitrite groups in paper VI. The correlation between the reduction in GI and increasing ammonia and nitrite levels suggests lower feed conversion efficiency in both groups. The combination of increasing mortality and no reduction in feed intake at relative low concentrations of unionised ammonia in paper VI illustrates that feed intake is not a good indicator of optimal conditions for gonad enhancement of adult green sea urchins. This result is supported by paper IV, where handling resulted in higher mortality levels but only a minor reduction in feed intake. Papers V and VI demonstrate that adult green sea urchins show relatively low tolerance to nitrite and un-ionised ammonia. Papers V and VI are the first reports to give the thresholds at which inorganic nitrogen influences gonad growth and feed intake of adult *S. droebachiensis*. The optimal conditions for gonad growth, including water quality parameters for nitrite and ammonia, are still not fully understood and require further research to understand the physiological mechanism involved in internal regulation of nitrite and ammonia of sea urchins. Water pollution can also stress the sea urchin and make it more vulnerable to diseases, e.g. bacterial infections (Böttger & McMlintock, 2009). New studies of sea urchins must also focus on whether high organic exposure can have a negative impact on the sea urchin’s immune defences.

5.2.3 Effects of different levels of oxygen on gonad growth and feed intake

Background

In water, the dissolved gases that are of particular biological and ecological importance are oxygen and carbon dioxide (Harmon, 2009). The solubility of dissolved gases in water depends on temperature, salinity and their individual partial pressure gradients across the surface (Timmens et al., 2001; Timmens & Ebeling, 2007).

Dissolved oxygen (DO) is often the single most important and critical water quality parameter in flow-through and recirculating aquaculture systems. When the level of DO in the water is low, feed intake may be suppressed. This is thought to be due to the reduced oxygen availability being unable to support the energy demands of the animal. A reduction in feed intake at low levels of DO would obviously have consequences for growth, and attempts have been made to determine critical levels of DO at which feed intake and growth become affected (Jobling, 1994). It is difficult to specify critical dissolved concentrations because the response to low DO is not life-or-death, but a continuum of physiological effects (Harmon, 2009). These effects are also influenced by the exposure time, the size and health of the animal, water temperature, concentration of carbon dioxide and other environmental conditions (Jobling, 1994).

It is well documented that a DO content below a critical level can reduce food consumption, growth rate and feed conversion efficiency in fish (Rankin & Jensen, 1993; Jobling, 1994; Wedemeyer, 1996; Thetmeyer et al., 1999; Foss et al., 2002). Sea urchins have no oxygen binding pigments in their coleomic fluids, and they lack a circulatory system. The water vascular system with the protruding tube feet constitutes their main means of external gas exchange (Steen, 1965). The water vascular system is internally lined by ciliated epithelium, which facilitates water movement inside the system. From the water vascular system oxygen has to pass a through a large coelomic space to reach the respiring tissue, which mainly consists of gonads and the digestive system. Even though the coelomic space is also lined by ciliated epithelium, this is not an efficient system for gas transportation (Johansen & Vadas, 1967).

Several studies have shown that the oxygen concentration in the coelomic fluid of sea urchins is directly related to the concentration of DO of the ambient water, and that the oxygen uptake also follows the DO. Thus, sea urchins are oxygen conformers and their oxygen uptake is restricted by insufficient oxygen supply (Giese et al., 1966; Johansen & Vadas, 1967; Webster & Giese, 1975; Spicer, 1995). The gonads of *S. droebachiensis* have a large capacity for aerobic metabolism (Percy, 1974). Still, Bookbinder & Shick (1986) have presented evidence that 76 – 92% of the heat dissipated by isolated ovaries from *S. droebachiensis* is derived from anaerobic energy metabolism. Evidence that gonads of sea urchins rely mainly on anaerobic metabolism also comes from Webster & Giese (1975), who measured very low oxygen concentrations, down to complete absence, in intact gonads of *S. purpuratus*.

Little information is available on the effects of hypoxia on gonad growth of sea urchins, and there are no published studies of long-term effects of hypoxia on adult sea urchins *S. droebachiensis*. Paper VII was, therefore, designed to investigate the long-term effects of reduced O_2 concentration on survival, gonad growth, and feed intake in the adult green sea urchin *S. droebachiensis*. The effects of oxygen content on gonad growth and feed intake in the green sea urchin, *S. droebachiensis*, were examined on adult sea urchins at oxygen levels of 4.0, 6.0 (hypoxia) and 9.5 mg l^{-1} (normoxia).

Gonad growth and total feed consumption at the end of the experiment were significantly higher in the control group (normoxia) compared with the hypoxic groups. Feed intake and total feed consumption were significantly reduced in the two hypoxic groups. The reduction in gonad growth was attributed both to a decrease in feed intake and impaired feed conversion ratio. Findings in paper VII suggest that adult green sea urchins have low tolerance to hypoxia, and are unable to maintain high gonad growth when exposed to such conditions.

These results support the findings of Spicer (1995) that sea urchins are unable to regulate their rate of oxygen uptake under hypoxia conditions. The lack of respiratory independence that characterises *S. droebachiensis* could be attributable to the fact that the sea urchin inhabits environments where oxygen stress is not really present. James (2007) found that water movement increased gonad growth in *E. chloroticus*, and attributed this to improved oxygen availability. These findings have led to the idea that it might be possible to increase growth in sea urchins beyond what has been achieved so far by supersaturation of the ambient water with oxygen. Support for this suggestion has already been presented since both Johansen and

Vadas (1967) and Webster & Giese (1975) have documented increased oxygen uptake in sea urchins offered super oxygenated water. An experiment by Mortensen & Siikavuopio (submitted) was undertaken in order to test the suggestion in *S. droebachiensis*, and includes examination of somatic growth of juveniles and gonad growth of adults kept in super oxygenated water. Juvenile and adult sea urchins were reared at constant oxygen levels of 100 (control), 115 and 130 percent oxygen, for respectively 42 and 50 days at 6.5 °C. The juvenile sea urchins in groups 100, 115 and 130 percent doubled their test diameter from and initial 11.5 mm to 19.9 mm, 21.4 mm and 20.6 mm respectively, but there were no significant differences between these groups. There were no significant differences in the GI, FI and FCR in any of the adult groups. Overall, our findings suggest that juvenile and adult green sea urchins can be reared at relatively high oxygen concentration over a long period, but are unable to utilise increased concentration of oxygen to increase somatic growth or gonad growth (Mortensen & Siikavuopio, submitted).

Oxygen supply has previously been shown to be a limiting factor for gonad growth in *S. droebachiensis* at saturation levels below 100% (paper VII). In paper VII, gonad growth was measured in sea urchins kept at 40, 60 and 100% oxygen saturation. These levels of saturation are low in order to establish a reliable correlation between oxygen saturation and gonad growth under hypoxic conditions in *S. droebachiensis*. However, the three points all fell on a straight line, indicating a linear relationship between oxygen saturation and gonad growth in this species. A nearly linear correlation between oxygen consumption and oxygen saturation has previously been found in another echonoid, the sand dollar (*Mellita quinquiesperforata* (Leske) by Lane & Lawrence (1979). Johansen & Vadas (1967), on the other hand, found that oxygen consumption reached a maximum level at about 50% oxygen saturation in *S. purpuratus*. Even though different parameters were measured in the experiments of Johansen & Vadas (1967) and Lane & Lawrence (1979) compared to Paper VII, the results still indicate that different species may react differently to differences in oxygen saturation of the ambient water. James (2007) found that gonad growth of *E. chloriticus* could be improved by keeping the animals in moving water, and suggested that this was due to improved oxygen availability. In line with this, Johansen & Vadas (1967) and Webster & Giese (1975) found increased oxygen consumption in *S. purpuratus* when offered oxygen enriched water.

The explanations of why the expected effects of oxygen enrichment did not appear in Mortensen & Siikavuopio (submitted) may be due to the reliance of sea urchin gonads on anaerobic metabolism. Only a thin surface layer receives sufficient oxygen to sustain aerobic metabolism (Bookbinder & Schick, 1986). It is a fair suggestion that increased DO in the coelomic fluid will allow oxygen to penetrate deeper into the gonads and support aerobic metabolism for a larger proportion of the gonads. Still, such an increase in aerobic capacity may be so marginal that the effect on gonad growth is negligible. It has also been suggested that gonad growth may be limited by supply of nutrients from the digestive system. If so, the gonads will not be able to benefit from higher oxygen availability unless the digestive system responds by increasing the supply of substrate for the gonad metabolism. The digestive system relies more upon aerobic metabolism than the gonads do and may already have reached its full capacity for food processing at normoxic conditions. Thus, there may be no more to gain by increasing ambient DO above this level.

Overall, these findings suggest that adult green sea urchins show low tolerance to hypoxia, and are unable to maintain high gonad growth under hypoxic conditions.

5.2.4 Effects of carbon dioxide on gonad growth and feed intake

Carbon dioxide is highly soluble in water, but the concentration in pure water is low (0.54 mg/L, at 20 °C) (Timmons et al., 2001). Most of the carbon dioxide in an aquaculture water column is the product of animal respiration and the decomposition of organic matter, with a small percentage coming from atmospheric diffusion (Timmons et al., 2001; Colt et al., 2009). Recirculating water systems with inadequate degassing can also be a source of high CO_2 levels (Timmons et al., 2001).

The concentration of carbon dioxide in water is determined by a gas-liquid equilibrium and also by series of acid-base reactions (Summerfelt, 2000). In fluids, dissolved CO_2 is hydrated to carbonic acid (H_2CO_3) and further to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). The proportion of each species of carbonate system (free carbon dioxide, bicarbonates and carbonate) depends on the H+ concentration of the water and, conversely, a change in these values will affect the pH. The acid-base reactions determine the chemical form in which dissolved inorganic carbon is present in water, and carbon dioxide concentration is thus primarily determined by the total amount of dissolved inorganic carbon present in water and of pH (Summerfelt, 2000; Colt et al., 2009).

Excessive CO_2 affects the fish by lowering blood pH and reducing the O_2-binding affinity of haemoglobin (Bohr and Root effects), thus interfering with the bloods ability to carry oxygen (Schmidt-Nielsen, 1997; Timmons, et al., 2001). High CO_2 levels can also increase the toxicity of ammonia (Johnston, 2001).

While there is abundant information on the effects of carbon dioxide in fish (Eddy et al., 1977; Heisler, 1984; Lemarie et al., 2000; Fivelstad et al., 2003; Foss et al., 2003; Martens et al., 2006), there have been no studies on the long-term effects of high carbon dioxide concentrations on gonad growth in sea urchins. The sea urchins need approximately 0.3 kg of calcium carbonate for every kilogram of weight they gain (Grosjean et al., 1998). However, urchins cannot assimilate carbonate from substrate, e.g. rocks, but can only absorb dissolved carbonates such as those present in the seawater. Therefore, in dense cultures there is a large depletion of carbonates from the seawater causing the pH to drop (Sim-Smith & Andrew, 2001; Colt et al., 2009). In order to avoid this, culture systems either need flow-through systems with a high water exchange rate or an adequate filtration system that removes CO_2 and carbon acid from the water. Paper VIII was, therefore, designed to investigate the effects of elevated carbon dioxide concentration on survival, gonad growth, feed intake, and feed conversion efficiency in adult *S. droebachiensis.*

In the experiment, adult sea urchins were exposed to two levels of carbon dioxide ($CO_{2\ (aq)}$), (1.1 mg l^{-1} (control) and 18 mg l^{-1}). Gonad growth and feed intake were both significantly reduced in the high concentration group compared to the control group. The reduction of about 50% in gonad growth and the threefold increase in feed conversion ratio indicates that the green sea urchin is more sensitive to elevated CO_2 than most finfish. A level of 15-20 mg CO_2 is recommended as steady state maximum for finfish in order to avoid negative effects on growth (Johnston, 2001; Timmons et al., 2001). In paper VIII, we suggested that the gonad growth was impaired by a lack of nutrients due to low feed intake, which in turn may be linked to a limited ability of digestive systems to process feed under such conditions. In paper VIII, two other explanations for the reduced gonad growth can be suggested: 1) The ability of the gonad tissues to convert substrate from the digestive system to gonad tissues is inhibited, or 2) the substrate is used for purposes other than gonad growth or a combination of both. Grosjean et al. (1998) listed problems that were likely to occur with further intensification of echinoculture, both in closed and semi-closed systems. These problems include accumulation of CO_2 in seawater in relation to skeletal growth. We did not observe somatic growth during this experiment, which may be due to the short duration of the experiment (paper III).

Grosjean et al. (1998) found that skeletal growth of the sea urchin *Paracentrotus lividus* was completely inhibited when the partial pressure of CO_2 in the water was increased five to nine times as compared to that of ordinary seawater. The test of sea urchins contains large amounts of calcium carbonate. Precipitation of bicarbonates into calcium carbonate is a dismutation reaction that liberates a stoichiometric amount of carbonic acid into the water column (Grosjean et al., 1998). Dissolved CO_2 in the water will thus push the equilibrium in the opposite direction, thereby inhibiting test growth. The observation that spines of the sea urchins from the high CO_2 group in paper VIII were more fragile than those from the control group is consistent with the idea that an elevated concentration of dissolved CO_2 may inhibit, or even reverse, the calcium carbonate formation in the sea urchin test. It is also likely that in an attempt to counteract this effect, sea urchins have to use energy for this purpose, which renders less energy for gonad growth. If this is true, it would lead to an increased FCR, as observed in the paper VIII. This implies that in evaluating threshold levels for dissolved CO_2 in sea urchin culture, measurement of skeletal growth is equally important as gonad growth and survival. In this study, we have not determined a tolerance level for dissolved CO_2 in sea urchin culture, but the current results indicate that *S. droebachiensis* may be more intolerant to elevated CO_2 levels than most fish species where guidelines for both cold and warm water species is $< 10\text{-}15$ mg l^{-1} (Wedemeyer, 1996).

5.3 Stocking density

Background

The development of long-term storage tank systems and roe enhancement techniques are needed in order to improve gonad quality and to obtain a higher value product. Determining the optimum stocking density is important for both economic and animal welfare reasons, as densely populated culture systems increase the risk of mortality and injury. To design a functional urchin holding system one must first understand some basics about sea urchin behaviour. Numerous aquaculturists and fishermen in Norway and the North Atlantic coastal areas have attempted to culture adult green sea urchins over the past decade (Devin, 2002, Aas, 2003). Urchin enhancers started with tanks designed to hold other species, e.g. Atlantic salmon. It was quickly learned that standard rectangular tanks are very inefficient for culturing sea urchins (personal observation). The sea urchins tend to cling to the walls and accumulate in corners, and even when there are more than sufficient surface areas, they will congregate and pile on top of another rather than distribute themselves along the bottom of the tank (Personal observation; Devin, 2002).

Based on these observations, the design of the tanks and stocking densities is very important. The literature includes some recommendations on optimal densities and maximal stocking densities of sea urchins (Le Gall, 1990). But the information and the database for these recommendations is not available (Le Gall, 1990). The results given by Le Gall (1990) seem, therefore, to be theoretical more than based on real experiments.

5.3.1 Effects of stocking density on gonad growth, feed intake and survival

In the papers IX and X, the effects of different stocking densities on survival, gonad index and feed intake of *S. droebachiensis* were examined in two different rearing systems: shallow raceways (paper X) and tanks with vertical lamellae (paper IX). In the paper X, in shallow raceways the green sea urchin performed well in raceways at the highest densities of 7-8 kg animal per m^2. In the paper IX, stocking density over 6 kg animals per m^2 had a significantly negative effect on gonad growth. The proportions of urchins with injuries also increased in relation to increasing stocking density.

The rearing system used in the paper IX appears to have a threshold density of 6 kg sea urchin m^2, below which mortality was negligible in tanks with vertical lamellae. Above the threshold, mortality increased rapidly with increasing stocking density. The explanation for the density-related mortality may be found in the behaviour of the animals. The sea urchins in the chambers with vertical lamellae were always sitting on vertical surfaces, except for short periods following feeding when all animals move down to the bottom of the tanks to pick up feed and thereafter return to positions at the vertical surfaces. It is likely that the negative interactions between the sea urchins, causing injuries and mortality, occurred during these vertical movements. This view is supported by the fact that the number of "collisions" between animals during the vertical movements would be positively correlated to the stocking densities.

If we assume that the occurrence of injuries and mortality are related to "collisions" between the sea urchins during vertical movements, stocking density *per se* may not be the only important factor. Most obvious is the fact that the number of "collisions" will also be determined by the height of the vertical surfaces on which the sea urchins climb. In the extreme case where the vertical surface is so low that there is only one row of sea urchins on it, there will be no need for the sea urchins to pass each other to gain feed, and there will probably be fewer interactions between the animals even at high stocking densities. Paper X supports the theory that the green sea urchin performs well in shallow raceways at the highest densities of 7-8 kg animal per m^2.

New studies on behaviour of sea urchins reported that the sea urchins migrated in random directions in the tanks until they met the food, thereafter staying at the same position until the food was eaten up (Miyamoto & Kohshima, 2006). Based on observation in papers IX and X and observations to Miyamoto & Kohshima (2006), the design of the tanks in relation to behaviour and stocking densities is very important to optimise the survival and growth. Further research on tank design is required. Another aspect of tank design is related to waste treatments (respiration, faces, waste feed) and corresponding water quality. From paper VII to X, we know that the urchins are sensitive to relatively small changes in water quality, especially ammonia. Use of , for example, long raceways with only one water inlet, can result in a water quality gradient with good water quality at one end of the raceway and poorer water quality the longer from the water inlet you come. These systems will increase the possible reduction in gonad growth and in worst-case scenarios lead to high mortality.

6 Summary and final considerations

In this thesis, the influence of a range of abiotic and biotic parameters on the survival, injury, gonad growth, feed intake and feed conversion ratio of adult *S. droebachiensis* has been discussed. The abiotic and biotic factors tested included: temperature, season, stocking density, handling and water quality parameters (oxygen, carbon dioxide, ammonia and nitrite). The main objective has been to identify some of the threshold limits where gonad growth performance is compromised and thus provide guidance for sea urchin farmers to maximise production. The focus of this thesis has been on the adult green sea urchin and it may not be possible to apply the results directly to other sea urchin species. Furthermore, the results are not recommended for juvenile *S. droebachiensis* because of possible ontogenetic differences in environmental tolerance and preferences.

Handling

Sea urchin gonad enhancement trials are prone to increased mortality during the first weeks after harvest. Controlled experiments have demonstrated that such post-harvest mortality is caused by a possible lack of salt water immersion and rough handling during harvest and transport. Rough handling may also cause non-lethal injuries which lead to reduced gonad growth due to a combination of reduced appetite and low feed conversion. Systematic studies on optimal treatment during harvesting and transport require further investigation.

Stocking density

Determining the optimum stocking density is important for economic and animal welfare reasons, as densely populated cultures reduced water quality and increases the risk of mortality and injuries. In the rearing system used in the experiments there appears to be a threshold density in relation to rearing systems. In cases where the vertical surface is low and there is only one row of sea urchins on it, there will be no need for the sea urchins to pass each other to gain feed, and there will probably be fewer negative interactions that can led to mortality and injury of the animals even at high stocking densities. This suggestion is supported by the stocking density experiment from the shallow raceways, where it was possible to keep higher stocking density (animals per m^2) compared to chambers with higher vertical surfaces. Based on these observations and the random moving behaviour of sea urchin, the design of the tanks in relation to behaviour and stocking densities is very important to optimise the survival and growth. Further research on tank design, feeding strategies and feeding systems (automatic systems) is required to improve survival, limit damage to the urchins and reduce the risk of negative interactions.

Temperature

This thesis outlines the importance of temperature on feed intake, gonad growth, and feed conversion ratio on *S. droebachiensis.* One important aspect of knowing the feed requirement in relation to different environmental conditions of the green sea urchin is that overfeeding is a major source of pollutants and can result in reduced water quality and thereby negative effects on gonad growth and survival of the green sea urchin in aquaculture, as illustrated in this thesis. Overfeeding has also an important implication for cost-effective feeding in aquaculture, since feed cost is one of the most important costs in aquaculture. Determining the optimum temperature and feed regime of the green sea urchin is, therefore, important in developing a sustainable environmentally friendly aquaculture industry.

The study has shown that the feeding rate increases with temperature and size of the urchins up to 14 °C. Findings suggest that optimal gonad growth of adult green sea urchins is achieved at higher temperatures in summer/autumn than in winter. The recommendation is that the adult green sea urchins should be reared at 10-12 °C in summer and at lower temperatures (under 10°C) in the winter in order to optimise gonad production. There is also an indication that there is a size dependent variation to temperature according to gonad growth and feed conversion ratio. At constant temperature, feeding rates of adult *S. droebachiensis* are strongly linked to season, being lowest under winter conditions and highest during summer. Further research on the effects of temperature in relation to gonad growth is required.

Water quality

Water treatment, temperature, stocking density or mortality at, for example, too high stocking densities are some of the factors that can affect the water quality in holding systems.

Our studies have illustrated that adult *S. droebachiensis* has a relatively low tolerance to increased levels of nitrite and ammonia. Our findings suggest that adult green sea urchins also show low tolerance to hypoxia and carbon dioxide, and are unable to maintain high gonad growth facing such conditions. *S. droebachiensis* has a low oxygen requirement (< 0.15 mg minute^{-1} kg^{-1} at temperatures < 14°C). But because they are unable to regulate their rate of oxygen uptake when the partial pressure of oxygen in the environment is reduced, they need relatively high water flow to maintain optimal gonad growth. Further investigations are required to point out more precisely water quality that does not affect gonad growth. The question should be addressed using a multifactor approach, which is far more effective at providing information that reflects a “true” culture situation, i.e. where several parameters interact and affect sea urchin performance simultaneously.

This results lead to the conclusion that monitoring water quality is critical when sea urchins are cultured under intensive conditions in order to avoid negative effects on productivity and animal welfare. The general impression is that *S. droebachiensis* requires good water quality in order to exhibit maximum gonad growth. Table 1 summarises the effects of different water quality on gonad growth, feed intake and mortality on adult sea urchins based on the findings in papers V, VI, VII and VIII.

Table 1: Different water qualities and the effects on gonad growth, feed intake (FI = g feed per animal per day) and mortality on *S. droebachiensis* .

Parameter	Standard	Temperature (°C)	Level	Gonad growth in relation to control group (100%)	Feed intake in relation to control group (100%)	Mortality (%)
O_2	mg/L	8	9.5	**100**	**100**	**15**
O_2	mg/L	8	6.0	**74**	**79**	**10**
O_2	mg/L	8	4.0	**68**	**69**	**15**
CO_2	mg/L	6	1.1	**100**	**100**	**5**
CO_2	mg/L	6	18.1	**50**	**83**	**3**
NO_2	mg/L	9	0.02	**100**	**100**	**0**
NO_2	mg/L	9	2	**85**	**98**	**0**
NO_2	mg/L	9	5	**75**	**96**	**0**
NO_2	mg/L	9	10	**69**	**98**	**0**
NH_3	mg/L	7	0.0001	**100**	**100**	**8**
NH_3	mg/L	7	0.016	**85**	**95**	**12**
NH_3	mg/L	7	0.032	**85**	**92**	**44**
NH_3	mg/L	7	0.064	**67**	**92**	**74**

To decide the oxygen requirement in flow-through systems for adult green sea urchins, we need first to know the oxygen consumption of the animal (paper I). In order to make a recommendation on the water requirement of adult *S. droebachiensis,* we also need to know the threshold level of hypoxic conditions where no negative effects occur in relation to gonad growth. We have not found these threshold levels (paper VI). It is, therefore, not yet advisable to set up correct water requirement for adult *S. droebachiensis.*

It is possible to reduce the water requirement by injection of oxygen, according to Mortensen & Siikavuopio (submitted). With design and operation of aeration systems, the impacts of carbon dioxide and un-ionized ammonia may hamper gonad growth. However, green sea urchins are extremely sensitive to organic exposure (Papers V-VIII) and injection of oxygen must be done with extreme care.

Optimum on-growing conditions, including optimal water flows, water quality parameters and tank design are still not fully understood. Technical bottlenecks are still posing challenges to the development of successful land-based holding systems for urchins.

The general impression is that *S. droebachiensis* has a strong requirement for water quality in order to exhibit a maximum gonad growth rate and survival. In natural ecosystems, the *S. droebachiensis* is found in a cold oxygen rich environment. Ongoing evaluation of optimal on-growing systems includes sea-based systems, and a prototype of a new commercial system for sea-based cultivation of sea urchins is currently being successfully tested in Northern Norway (e.g. the Seanest system, www.seanest.no). Based on the results of this thesis and the general lack of knowledge, my recommendation for future sea urchin holding system design would be to avoid intensive recirculation systems and to focus on using land-based flow-through raceway-systems or sea-based systems.

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Part Two

List of Papers

This thesis is based on the following papers, referred to by their Roman numbers:

I. Siikavuopio, S. I., Mortensen. A., Christiansen, J. S., 2008. Effects of body weight and temperature on feeding, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*. **Aquaculture** 281, 77-82

II. Siikavuopio, S. I., Christiansen, J.S., Dale, T. 2006. Effects of temperature and season on gonad growth and feed intake in the green sea urchin (*Strongylocentrotus droebachiensis)*. **Aquaculture** 255, 389-394.

III. Siikavuopio, S. I., Christiansen, J. S., Sæther, B-S., Dale, T., 2006. Seasonal variation in feed intake under constant temperature and natural photoperiod in the green sea urchin (*Strongylocentrotus droebachiensis)*. **Aquaculture** 272, 328-334.

IV. Dale T., Siikavuopio, S. I., Aas, K., 2005. Roe enhancement in sea urchin: effects of handling during harvest and transport on mortality and gonad growth in *Strongylocentrotus droebachiensis*. **Journal of Shellfish Research** NO.4, 24, 1235-1239.

V. Siikavuopio, S. I., Dale, T., Foss, A., Mortensen, A., 2004. Effects of chronic ammonia exposure on gonad growth and survival in green sea urchin, *Strongylocentrotus droebachiensis.* **Aquaculture** 242, 313-320.

VI. Siikavuopio, S. I., Dale, T., Christiansen, J. S., Nevermo, I., 2004. Effects of chronic nitrite exposure on gonad growth in green sea urchin, *Strongylocentrotus droebachiensis.* **Aquaculture** 242, 357-363.

VII. Siikavuopio, S. I., Dale, T., Mortensen, A., Foss, A., 2007. Effects of hypoxia on feed intake and gonad growth in the green sea urchin (*Strongylocentrotus droebachiensis*). **Aquaculture** 266, 112-116.

VIII. Siikavuopio, S. I., Mortensen, A., Dale, T., Foss, A., 2007. Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin (*Strongylocentrotus droebachiensis*). **Aquaculture** 266, 97-101.

IX. Siikavuopio, S. I., Dale, T., Mortensen, A., 2007. The effects of stocking density on gonad growth, survival and feed intake of adult green sea urchin (*Strongylocentrotus droebachiensis*). **Aquaculture** 262, 78-85.

X. Christiansen, J. S., Siikavuopio, S. I., 2007. The relationship between feed intake and gonad growth of single and stocked green sea urchin (*Strongylocentrotus droebachiensis*) in a raceway culture. **Aquaculture** 262, 163-167.

Paper I

Paper II

Paper III

Paper IV

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Paper VII

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Paper X