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METABOLIC OXYGEN REQUIREMENTS OF SMALL, PLEURONECTID FLATFISH UNDER SIMULATED AQUACULTURE CONDITIONS.

A Thesis
Submitted to the Graduate Faculty
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science
in the Department of Pathology and Microbiology
Faculty of Veterinary Medicine
University of Prince Edward Island

Paul F. MacIsaac
Charlottetown, PEI
October, 1996

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ABSTRACT

The metabolic requirements of small, pleuronectid flatfish were determined by measuring routine oxygen consumption (ROC). ROC rates were measured using single-pass, flow-through respirometry tanks designed to simulate land-based aquaculture holding conditions. The influence of each test parameter was affected by temperature. Interactions between test parameters and oxygen consumption rate were significant according to ANOVA ($P<0.05$). In the first experiment the highest ROC rates (mg O_2 • kg^{-1} • h^{-1}) of yellowtail flounder, *Pleuronectes ferruginea*, and winter flounder, *Pleuronectes americanus*, followed a parabolic response over the temperature range tested (2 - 14°C). Peak metabolism was at approximately 11°C. The ROC rates of American plaice, *Hippoglossoides platessoides*, did not follow a parabolic response to increasing temperatures. In the second experiment winter flounder weighing 75 - 455 g had decreased ROC (mg O_2 • kg^{-1} • h^{-1}) as body size increased. The decrease in oxygen consumption over the size range tested over three temperatures was best described with a linear equation where $MO_2 = 118.9 + 1.05(T) - 0.16(W) + 14.3(R) - 22.1(C)$. W was mean fish size (g), T was temperature (°C), R was trial and C was tank. In the third experiment the ROC rates of winter flounder were determined at three stocking densities (5 kg • m^{-3}, 25 kg • m^{-3}, 50 kg • m^{-3}) and repeated at 2.2 and 9.7°C. Oxygen consumption and stocking density were inversely related as stocking densities approached 50 kg • m^{-3}. At 2.2°C, the highest ROC rate was observed in the 5 kg • m^{-3} density group (50.4 ± 31.9 mg O_2 • kg^{-1} • h^{-1}) and lowest in the 50 kg • m^{-3} density group (28.5 mg O_2 • kg^{-1} • hr^{-1}). At 9.7°C the highest oxygen consumption rate was observed in the 25 kg • m^{-3} density group (60.9 ± 13.2 mg O_2 • kg^{-1} • h^{-1}) and the lowest in the 50 kg • m^{-3} density (44.2 mg O_2 • kg^{-1} • hr^{-1}). These data provide the minimum aerobic requirements of flounder during the on-growing phase of production, and suggest that intensive on-growing of these fish may be economically viable.

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Finally a special acknowledgement to Christy, my best friend, best of luck.

To my father; whose own enthusiasm to grow fish inspires my own.

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LIST OF ABBREVIATIONS.

a	- y-intercept
ADP	- adenine-5'-diphosphate
AGMP	- Atlantic Groundfish Management Plan.
∝	- alpha
AMP	- adenine-5'-monophosphate
ANOVA	- analysis of variance
ATP	- adenine-5'-triphosphate
b	- slope
BOC	- bacterial oxygen consumption
C	- tank
°	- angular degrees
°C	- degrees Celsius
DF	- degrees of freedom
DO	- dissolved oxygen
EMG	- electromyogram
F	- F-value
FAO	- Food and Agriculture Organization of the United Nations.
g	- grams
GE	- group effect
>	- greater than
h	- hour
kg	- kilograms
L	- litres
m	- metre
M	- biomass of fish
μm	- micrometers
mg	- milligrams
min	- minutes
mL	- millilitres
mm	- millimetres
MO_2	- oxygen consumption rate
M_{1MO2}	- oxygen consumption rate of density group 1.
M_{2MO2}	- oxygen consumption rate of density group 2.
N	- sample size
N.B.	- New Brunswick
O_2	- oxygen molecule
$[O_2]_F$	- final dissolved oxygen concentration
$[O_2]_I$	- initial dissolved oxygen concentration
$[O_2]_{in}$	- dissolved oxygen concentration of inflowing water
$[O_2]_{out}$	- dissolved oxygen concentration of outflowing water
P	- probability
pH	- hydrogen ion concentration
%	- percent
r^2	- Pearson correlation coefficient of determination
R	- trial
rbc	- red blood cell
ROC	- routine oxygen consumption
SA	- surface area
SAS	- Statistical Analysis Software
sec	- second
s.d.	- standard deviation
s.e.m.	- standard error of the mean
SDA	- specific dynamic action
SNK	- Student Newman-Kuels non-parametric test
T	- temperature
V	- volume
V_w	- water flow rate
W	- body weight

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1.0 GENERAL INTRODUCTION.

1.1 Current State of Flatfish Aquaculture.

Fish farming is a young, growing industry, which is beginning to diversify in Atlantic Canada (Brown et al. 1995, Brown 1994). Although Atlantic salmon (*Salmo salar*) is the most valuable finfish species cultured in this region, several other marine species are being considered for their culture exploitation in the Atlantic Canada marine environment.

1.1.1 Flatfish Culture.

Two flatfish species that are cultured successfully are the Japanese flounder (*Paralichthys olivaceus*) and the European turbot (*Scophthalmus maximus*). Japan cultures more than 10,000 metric tonnes of flounder valued at $146,000,000 US in 1993 (FAO 1995). Its high market value in Japan is due to the quality of its white flesh (Ikenoue and Kafuku 1992).

Turbot is considered a luxury fish on both French and Spanish markets (Person-LeRuyet 1990). In 1993, production was greatest in Spain with production of 2000 metric tonnes and value $20,000,000 US (FAO 1995).

Atlantic halibut (*Hippoglossus hippoglossus*) is a cool water species of interest for aquaculture in Europe and North America. Although it is a high quality fish production is experimental (Brown and Keough 1994, Tilseth 1990, Tilseth et al. 1992). Metamorphosed halibut were first produced in Norway in 1985 (Brown and Keough 1994) and by 1993 140,000 juvenile halibut were produced in Norway and about 2000 halibut fry in Canada, Iceland, and Scotland combined (Björnsson 1994).

1.1.2 Other Aquaculture Species.

The potential benefit of diversification in Canadian aquaculture is widely recognized (Waiwood et al. 1988). Bluefin tuna (*Thunnus thynnus*), Atlantic cod (*Gadus morhua*), Atlantic halibut, and lumpfish (*Cyclopterus lumpus*) have been considered as potential species for exploitation on the East coast of Canada. Currently, laboratory research in Canada on culture technology has begun on several other species including Atlantic halibut, striped bass (*Morone saxatilis*), winter flounder (*Pleuronectes americanus*), yellowtail flounder (*Pleuronectes ferruginea*) and two wolffish species, (*Anarhichas lupus*) and (*A. minor*).

1.1.2.1 Flatfish Candidates for Aquaculture.

American plaice (*Hippoglossoides platessoides*), witch flounder (*Glyptocephalus cynoglossus*), and yellowtail flounder have comprised large portions of Canada's groundfish catch, second only to cod (Bowering and Brodie 1991). In the 1960's and 1970's, catches of American plaice surpassed 90,000 tonnes (Pitt 1975), but competition by foreign countries fishing outside the 200-mile boundary severely affected fish stocks (Walsh 1991). American plaice and yellowtail flounder have been under quota restrictions since 1974 (Bowering and Brodie 1994); quotas were eliminated in 1994 (AGMP 1994).

Yellowtail flounder, winter flounder, and American plaice share several characteristics of ideal aquaculture candidates. These fish have strong market value in Canada, the United States, and Europe. Decreased landings by the wild fishery have resulted in a shortfall in supply of flatfish products. Developing the ability to supply markets with fresh flatfish products of consistent quality on a regular schedule will ensure a good return.

1.1.2.2 Biology of Candidate Flatfish.

American plaice, winter flounder, and yellowtail flounder are native to the waters of the Northwest Atlantic (Scott and Scott 1988, Bigelow and Schroeder 1953). Distributions of yellowtail flounder are governed by bottom type (Scott 1982) and water depth (Bowering and Brodie 1991, Colton 1972), whereas American plaice and winter flounder respond to seasonal changes in temperature (VanGuelphan and Davies 1979, Pitt 1967, Powles 1965, McCracken 1963) and food availability (Kennedy and Steele 1971). American plaice have the coldest water temperature distribution compared to the other two fish. The optimal temperature range of American plaice spans -0.5 - 2.5°C (Bowering and Brodie 1991), whereas the optimal temperature range of yellowtail and winter flounder spans 2 - 12°C (Bowering and Brodie 1991, Scott and Scott 1988, Colton 1972, McCracken 1963). Flounder survive sub-freezing winter temperatures by producing an anti-freeze protein in their blood serum (Davies et al. 1988).

Maturation has been directly related to fish length rather than age in these three species (Bowering and Brodie 1994, Kennedy and Steele 1971, Pitt 1971, Topp 1968, Pitt 1964). Males mature before females and at a smaller size (Bowering and Brodie 1991, Miller et al. 1991). The spawning season is regulated by water temperature (Bowering and Brodie 1994, Neilson et al. 1988, Pitt 1966) with spawning occurring throughout the spring and early summer at water temperatures of 2 - 8°C (Frank et al. 1992, Harim and Crim 1992, Neilson et al. 1988, Scott and Scott 1988, Powles 1965).

Eggs are spherical and small (yellowtail and winter flounder - 0.8-0.9 mm diameter, American plaice - 1.5-2.8 mm) (Scott and Scott 1988). Winter flounder eggs are adhesive and demersal (Chambers and Leggett 1987) whereas yellowtail and American plaice eggs are buoyant

(Scott and Scott 1988). Eggs will hatch within one or two weeks depending on the temperature (Litvak 1994, Scott and Scott 1988). Larvae are pelagic, only becoming benthic after metamorphosis (Fahay 1983, Pitt 1967). Metamorphosis occurs 40-80 days post-hatch at 8°C in winter flounder (Litvak 1994, Chambers and Leggett 1987, Fahay 1983) and 50-80 days post-hatch at 6°- 8°C in yellowtail flounder (J.A. Brown, Ocean Sciences Center, Memorial University, pers. comm.).

These three flounder species are generally invertebrativorous in the wild (Libey and Cole 1979, Kennedy and Steele 1971, Powles 1965). The specific composition of the diet of each species includes common and separate prey items. Copepods, crustaceans and polychaete worms are commonly eaten (Collie 1987, Langton 1983, Libey and Cole 1979, Kennedy and Steele 1971). These flatfish rely on visual acuity to forage, which occurs most intensively during afternoon-evening periods for the deeper species (Collie 1987, Langton 1983, Libey and Cole 1979, Powles 1965) and following flood tides for winter flounder (Kennedy and Steele 1971, Tyler 1971). Competition from other benthivorous feeders such as Atlantic cod is significant in wild populations of American plaice (Pitt 1967, Powles 1965). Diets for larval flounder include zooplankton, nauplii, and small polychaetes (Litvak 1994, Pearcy 1962) which have been elucidated from artificial culture experiments.

1.2 Respirometry.

1.2.1 Respiratory Physiology and Application to Aquaculture.

Aquaculture is ultimately a industry that benefits from several scientific disciplines including physiology, ecology, animal behavior and engineering. One area of research that is crucial to the aquaculture industry is metabolism. Studies focusing on metabolism have associated growth to exercise (Brett 1964), growth to temperature (Fonds et al. 1992), growth to stocking density (Kjartansson et al. 1988), and survival to behaviour (Metcalfe et al. 1995). Other studies of metabolism have addressed the metabolic effects of stress (Barton and Schreck 1987), and the water flow requirements for land based aquaculture enterprises (Sigholt et al. 1993, Fivelstad and Smith 1991, Fivelstad 1988).

To develop new aquaculture technologies with reasonable production costs, an understanding of the factors which promote survival and growth are essential. For example, Davenport et al. (1990) studied differences in gut transit, appetite, oxygen uptake and nitrogen excretion between wild caught Atlantic halibut and Lemon sole (*Microstomus kitt*) held in duoculture (two species grown within one environment). Both halibut and lemon sole have low rates of oxygen uptake and nitrogen excretion. Their feeding behaviours complement each other in duoculture. Such studies measure the metabolism of fish reared in an artificial environment, and are useful when designing production systems.

1.2.2 Why Measure Oxygen Consumption?

In fish aerobic energy is obtained from the hydrolysis of adenine-5'-triphosphate (ATP) to adenine-5'-diphosphate (ADP) and adenine-5'-monophosphate (AMP) (Lehninger et al. 1993). Hydrolysis requires oxygen molecules from tissue cells. The supply of oxygen in the tissues is finite and is always spent and refueled.

Fish obtain oxygen from the external environment through counter-current gas exchange at the gills (Olson 1991). Oxygen is transported to the tissues by the blood (Olson 1991). Oxygen follows an oxygen gradient while in the blood stream, exiting wherever the gradient is strong enough to shift oxygen molecules from the blood stream to the tissues (Eckert et al. 1988).

One example of how oxygen is used for energy is during growth. Following ingestion of nutrients, rates of oxygen consumption are elevated for several, depending on temperature, for digestion (Jobling and Davies 1980). During digestion, an influx of amino acids into the blood apparently increases the rate of protein synthesis and metabolism (Jobling 1994). The increased energy demand after feeding is necessary to maintain homeostasis. However, fish grow as a result of feeding, implying that some component of the energy supply is used for metabolic functions and the remainder is utilized during protein synthesis for body mass (Jobling 1994).

The requirement for oxygen during physiological functions demonstrates the importance in measuring the consumption of oxygen by fish. Such a procedure is a reliable means of estimating their energy use (Cech 1990), and additional to fundamental understanding of bioenergetics, estimates of oxygen consumption are valuable in assessing water flow requirements for culture operations (Fivelstad 1988).

1.2.2.1 Why Study the Physiology of Three Flatfish Species?

The oxygen consumption rates of all flatfish are lower than those of other teleosts such as salmonids (Waller 1992, Fivelstad and Smith 1991, Davenport et al. 1990, Brown et al. 1984, Jobling 1982). In intensive, land-based aquaculture lower metabolic demands should have economic benefit. A good flatfish species for commercial exploitation is being sought; applying the comparative approach to determine oxygen requirements is a logical process in choosing a suitable species to culture.

1.2.3 What is Respirometry?

Metabolic rates of animals are determined by two calorimetric methods (Cech 1990, Fry 1971). Measuring heat production by direct calorimetry is useful in homeotherms. However, poikilothermic fish produce low amounts of heat that are readily dissipated to the aqueous environment; accurate results are difficult to obtain using direct calorimetry.

Respirometry is an indirect method of determining metabolic rate by measuring the rate of oxygen consumption. It is useful in modelling the physiological requirements of fish and the scope of growth and production under varying conditions inherent to aquaculture.

1.2.4 Considerations of Respirometry.

During respirometry, many variables influence the collection of reliable and repeatable data (Brett 1962). Variables which mask results when not controlled include the time required by fish to acclimate to the laboratory and respirometer, the holding conditions, and feeding routine of the fish.

Acclimation time is required after moving fish because handling stress elevates oxygen consumption rates (Fry 1971) and increases lactic acid levels (Beamish and Mookherjii 1964). This period facilitates the return to baseline physiological conditions.

Acclimation also permits digestion and evacuation of gastrointestinal contents (Jobling 1980). In respirometry experiments, energy required for digestion masks the energy used for metabolism (Jobling 1980, Jobling and Davies 1980). The period of increased oxygen consumption during digestion is defined as specific dynamic action (SDA)(Jobling and Davies 1980). Excretory products from fish support bacterial cultures which can compete for available oxygen (Jarboe 1995, Tanaka and Kadowaki 1995, Jobling and Davies 1980). Fasting fish for 24 h prior to moving fish into a respirometer reduces the probability of an acute response to the increased stress of handling (Beamish and Mookherjii 1964, Fry 1971), likely because additional energy required during digestion is not dominating the use of available energy (Jobling 1980, Jobling and Davies 1980). Holding fish in the respirometer for 12 - 16 h before beginning measurements allows fish to acclimate to the chamber and gives more reliable data (Jobling 1982).

1.2.5 States of Oxygen Consumption.

A critical issue in fish respirometry studies is the activity state of the fish. The three categories of fish activity include (i) standard or resting metabolism, (ii) resting routine and routine metabolism, (iii) swimming and active metabolism (Cech 1990, Fry 1971).

(i) Standard metabolism.

Standard metabolism is the amount of energy required to exist when no random activity occurs. Beamish and Mookherjii (1964) defined standard metabolism as the closest approximation to the basal metabolism obtained when all organs were absolutely at rest. For standard metabolism, the fish should be totally quiescent and should not be disturbed by any external or internal stimuli.

(ii) Resting routine and routine metabolism.

Cech (1990) described resting routine metabolic rate as the range of activity between standard and active metabolism. It is the minimum energy required over a 24 hour cycle. Resting routine metabolism attempts to measure oxygen consumption in the quiescent animal over an extended period (Cech 1990, Fry 1971). The fish should not have food in its digestive tract.

Swimming should be limited to no more than 1 bodylength • sec^{-1} (Cech 1990). Routine metabolism involves daily cycles of activity in the form of spontaneous swimming spurts (Forstner 1983). Most studies of fish energetics are conducted using routine metabolism (Beamish and Mookherjii 1964, Forstner 1983).

(iii) Swimming and active metabolism.

Swimming metabolism is determined for a fish in sustained swimming, though not at maximum swimming capacity. In comparison, active metabolism is established by inducing the highest sustainable swimming speed and determining the maximum oxygen consumption rate (Cech 1990, Fry 1971, Beamish and Mookherjii 1964). A respirometer that permits the fish to swim against a consistent laminar flow is required (Brett 1964).

Comparing the standard rate of oxygen consumption of a fish versus an active rate of oxygen consumption is not valid. An appropriate respirometer must be used according to the scale of activity. The size and structure of the respirometer may affect the behaviour of the animal, which can affect its oxygen consumption rate (Forstner 1983).

1.2.6 Respirometers.

Two types of respirometers are used to determine metabolic rate: static systems and flow-through systems. Static systems utilize a fixed volume of water from which oxygen is consumed over a short duration (Cech 1990, Beamish and Mookherjii 1964). Flow-through systems have continuous water flowing over the fish in the respirometer and experiments are for longer periods.

1.2.6.1 Static Respirometers.

The earliest studies of metabolic requirements of animals using oxygen consumption involved placing animals in sealed containers and measuring the rate of oxygen depletion over a short interval (Zeuthen 1953). Oxygen consumption rate was then calculated as (Cech 1990):

$$MO_2 = \frac{\{[O_2]_I - [O_2]_F\} \bullet V}{h}$$

where MO_2 is oxygen consumption rate (mg $O_2 \bullet h^{-1}$), $[O_2]_I$ is the oxygen concentration in water (mg $O_2 \bullet L^{-1}$) at the start of the trial, $[O_2]_F$ is the oxygen concentration in water (mg $O_2 \bullet L^{-1}$) at the end of the trial, V is the volume of the respirometer (L), and h is the duration of the experiment (hours). All states of oxygen consumption can be estimated using this technique, either by inducing fish to swim before placing them in the respirometer for active metabolism, or by plotting oxygen consumption rates at various swimming speeds and extrapolating to no swimming activity to obtain an estimate of standard metabolism (Beamish and Mookherjii 1964, Brett 1964).

In static tanks, the accumulation of waste products and bacterial oxygen consumption (BOC) may affect the accuracy of the experiment (Cech 1990, Bishop 1976). These substances compete with fish for available oxygen (Bishop 1976). A "blank" respirometer running in parrallel with the experimental trial corrects for BOC (Cech 1990, Bishop 1976). However, different bacteria are present in the water supply other than those native to the skin of fish and on waste products (Dalla Via 1983). Other compensations for microbial oxygen consumption are (i) adding antibiotics and (ii) measuring oxygen consumption of the empty test chamber after an experimental period has been completed (Graham and Baird 1984). Both are problematic since antibiotics add a new parameter to the test and removing fish from the test chamber leads to the introduction of atmospheric air. The effect of BOC must be considered carefully with respirometry work, and there is no standard protocol to follow to completely control BOC.

Tank volume, specifically the ratio of tank size to biomass, is also important (Cech 1990). Fonds et al. (1992) used static systems to measure the difference in oxygen consumption of two species of flatfish of different sizes, at different temperatures and different states of satiation. The size of the fish is important because the oxygen saturation in the water bath is fixed and should not fall below 30% saturation. Otherwise, a change in the respiratory behaviour of the fish is stimulated (Fry 1971, Voyer and Morrison 1971). Cech (1990) advised that respirometry chamber volumes should be 30-50 X greater than the size of the fish to avoid a hypoxic environment. Marty et al. (1995) used static 10 and 20 mL glass syringes to measure the oxygen consumption of Japanese medaka (*Oryzias latipes*) larvae. The larvae were small enough to survive in the syringes for five days; thus determining oxygen consumption rate was reliable. However, many aquatic organisms are oxygen dependent in low oxygen saturation and consequently, true oxygen consumption rates do not result from such a respirometry test (Bishop 1976).

1.2.6.1.1 Swimming Respirometers

Swimming respirometers incorporate principles of both static and flow-through respirometers. Fish are induced to swim by a recirculating flow at a fixed rate of water propulsion (Cech 1990, Brett 1964). Oxygen consumption is estimated by turning the water flow off and measuring the depletion of oxygen over time (Cech 1990, Fry 1971, Brett 1964). Oxygen consumption is then correlated to swimming velocity (Cech 1990, Brett 1964).

Classic swimming respirometers were used by Brett (1964) and Blazka (Blazka et al. 1960). Brett's respirometer recirculated water, maintaining slightly higher than ambient dissolved oxygen (DO) in the reservoir water and at atmospheric pressure to avoid supersaturation. Water, 100% oxygen saturated, was pumped into the chamber continuously through the system to maintain a strong flow against the fish. In Brett's experiments, if the oxygen saturation in the system decreased below 70% ambient saturation during an experiment, measurements were stopped and the chamber was flushed with freshly aerated water (Brett 1964).

Priede and Holliday (1980) modified a Brett respirometer to determine the standard and active oxygen consumption rates of flatfish by designing a chamber which tilted to induce the fish to swim. With the respirometer at 0° of inclination, standard metabolism could be measured. When partial pressures of oxygen fell below 80% of full saturation, the tank would be flushed with aerated water from the header tank. Active oxygen consumption was measured by tilting the chamber 30 - 50° which then induced the fish into sustained swimming (Priede and Holliday 1980). Grottum et al. (1993) combined the swimming chamber of Brett (1964), used for fusiform fish, with the swimming chamber of Priede and Holliday (1980). Both fusiform fish and flatfish could lie at rest like that of Priede and Holliday (1980) plus the ellipsoid-shaped chamber could be tilted to induce swimming in flatfish.

The Blazka respirometer is modelled after a 'tube within a tube' design (Blazka et al. 1960). Inside a larger tube was the swimming chamber where fish were placed. Water was pumped through the inner 'tube' past the fish, and then recirculated through the outer tube back to the front of the unit. Construction of the end of the tube and placement of radial plates in the front were important to insure constant laminar flow through the system. Speed of the impeller at the front of the outer tube regulated the swimming speed of the fish.

Weatherley et al. (1982) used an unique method to measure metabolism without measuring oxygen concentration. This procedure determined metabolic rates of rainbow trout, *Oncorhynchus mykiss*, at different swimming speeds by recording bioelectrical signals emitted by myomeres. The bioelectrical signals were read from an electromyogram (EMG) and correlated to the heart rate of the fish, which was then used to determine oxygen consumption rate. Routine, spontaneous, and forced swimming metabolism were measured without confining the fish to small measurement chambers.

1.2.6.2 Flow-Through Respirometers.

Flow-through respirometers have certain advantages over static respirometers. Flow-through respirometers permit longer studies to be conducted. A better indication of the true metabolic rate of the animal is possible because fully saturated water is always available to the fish (Bishop 1976).

In flow-through designs, DO entering and leaving the respirometer and the water flow rate must be measured (Cech 1990). Cech (1990) described an equation, under stable conditions, to calculate oxygen consumption rate in a flow-through respirometer:

$$MO_2 = [O_2]_{in} - [O_2]_{out} \bullet Vw;$$

where MO_2 is the oxygen consumption rate in mg $O_2 \bullet min^{-1}$, $[O_2]_{in}$ is the oxygen concentration of inflowing water (mg $O_2 \bullet L^{-1}$), $[O_2]_{out}$ is the oxygen concentration of outflowing water (mg $O_2 \bullet L^{-1}$), and Vw is the water flow rate through the respirometer ($L \bullet min^{-1}$).

Bishop (1976) designed a flow-through respirometer with an aerated header tank which flowed by gravity to the respirometer. The oxygen saturation level in the header tank could be regulated to a specific saturation to test the effect oxygen saturation had on fish oxygen consumption. Caulton (1978) also used this flow-through design to determine the effect of body mass and temperature on the oxygen consumption of larger (10 - 150 g) tilapia (*Sarotherdon mossambicus*), which previously had only been determined using static respirometers. Sigholt et al. (1993) determined the effect feeding had on the routine oxygen consumption of juvenile cod (*Gadus morhua*) and developed a model which incorporated temperature and fish weight for fed and unfed fish using a design similar to those of Bishop (1976) and Caulton (1978).

Cech et al. (1979) fitted a small, plexiglass chamber inside a larger tank for use as a flow-through respirometer. The larger tank was filled with water to create a constant temperature bath. Using this design, Cech et al. (1979) determined that 30-40 hours after handling, the resting metabolism of largemouth bass (*Micropterus salmoides*) returns to normal levels. This was affected by experimental temperature and dissolved oxygen. This respirometer was also used to determine the effect of acclimation temperature and body mass on the standard oxygen consumption of Northern squawfish (*Ptychocheilus oregonsis*)(Cech et al. 1994). A logarithmic relationship described the trend of increasing temperature and increasing oxygen consumption, whereas oxygen consumption decreased per unit body weight as fish got larger.

Jobling and Davies (1980) used a flow-through respirometer to determine the standard metabolism of flatfish and the energy requirements for specific dynamic action after a feeding interval. An 80% oxygen saturation in the outflowing water was held constant by adjusting flow rates from a header tank. Similarly, Ross and McKinney (1988) determined the standard oxygen consumption of Nile tilapia (*Oreochromis niloticus*) through multiple diel cycles, indicating much of the variation in the data could be accounted for by light and dark periods.

Several authors have illustrated the effects of environmental stressors, such as increased temperature and hypoxia (Peterson and Ardahl 1992, Cech et al. 1979, Kerstens et al. 1979, Cech et al. 1977, Cech et al. 1976, Voyer and Morrison 1971), and social stressors, such as aggressive behaviour (Metcalfe et al. 1995, Brafield and Matthiessen 1976), on oxygen consumption. Production stressors, such as increased stocking density (Miller et al. 1995, Staurnes et al. 1994) and increasing toxicities (Brafield and Matthiessen 1976), are effectively determined using flow-through respirometry because a controlled environment is maintained (Caulton 1978).

However, studies of the responses to environmental stressors is limited by the lag time between the treatment and the response (Steffensen 1989). The measured response of a fish to a stressor will be delayed depending on the turnover rate in the tank, or the lag time (Steffensen 1989). If the measurement period is shorter than the lag time, the response by the fish to a stressor will not be determined (Niimi 1978).

1.3 Fish Respiration and Metabolism.

In aquaculture, maximizing growth is the objective of understanding metabolism. Growth can be influenced by physical factors such as temperature (Hoornbeek et al. 1982), stocking density (Kjartansson et al. 1988), oxygen level (Bejda et al. 1992), salinity and photoperiod (Jobling 1994). Aerobic metabolism is influenced by the same factors, plus somatic factors, such as fish size, maturity, and nutritional status (Jobling 1982, Jobling 1980, Brett and Zala 1975, Beamish 1964a). To maximize fish performance, oxygen transport through fish and the effect of the rearing environment has on oxygen consumption must be understood.

1.3.1 Oxygen Exchange and Transport.

The availability of oxygen to organisms in the aquatic environment is 26X less than the oxygen available to terrestrial organisms (Davis 1975). Increasing temperature and salinity further reduce solubility of oxygen in water, thereby reducing maximum oxygen saturation (Davis 1975). Fish take oxygen through gills; heavily vascularized tissues that allow deoxygenated blood to comes into close contact with the outside environment and reoxygenate (Perry and McDonald 1993).

Oxygen binds to red blood cells (rbc's) because rbc's contain haemoglobin, an iron-rich molecule, has high affinity for oxygen when oxygen pressure is high (Satchell 1971). This phenomenon is the Bohr effect, the dependence of oxygen saturation of haemoglobin on hydrogen ion concentration (Bartels et al. 1973). Oxygen contacts haemoglobin by simple diffusion across a gradient established by hydrogen ions. The oxygen molecule passes through the membrane of gill lamellae and through the membrane of the rbc before contacting haemoglobin (Eckert et al. 1988). During systemic circulation, oxygen is released from haemoglobin to the tissues due to low oxygen pressure (Perry and McDonald 1993, Satchell 1971).

1.3.2 Factors Influencing Oxygen Consumption.

1.3.2.1 Somatic Factors.

Many somatic and physiologic factors influence oxygen consumption rates. These include fish size (Jobling 1982, Laurence 1975), maturity (Beamish 1964a), stress (Barton and Schreck 1987), feeding level (Jarboe 1995) and digestion (Brett and Zala 1975).

Fish undergo allometric growth; poorly vascularized muscular tissue represents a larger portion of the total mass than metabolically active tissue like the gastrointestinal tract and liver (Eckert et al. 1988). This results in decreasing oxygen consumption per unit of body weight as fish grow larger (Jobling 1994).

The allometric relation for rate of oxygen consumption and increasing body weight is

$$MO_2 = a\,W^{b}$$

where MO_2 is the rate of oxygen consumption ($mg\ O_2 \cdot h^{-1}$), W is body weight (in grams), a is a temperature-dependent constant and b represents the slope of the equation (Jobling 1994, Fonds et al. 1992, Jobling 1982). Studies determining the effect of body size on oxygen consumption in fish are numerous. Estimates of the weight exponent (b) have ranged from 0.65-0.9, depending on sizes of fish studied and species (Jobling 1994, Cech 1990, Brett 1962). Fish size at various stages of the production cycle is an important consideration to ensure an adequate supply of oxygen is available.

The three states of activity influence oxygen consumption by increasing metabolism associated with increased swimming (Webb 1971). The relationship between swimming speed and oxygen consumption rate is positive up to a critical swimming speed where fatigue slows the activity of the fish (Preide and Holliday 1980, Webb 1971, Brett 1964).

After feeding, metabolic oxygen requirements increase, reflecting energy demands for digestion (Jobling 1980). Oxygen consumption rates are higher post-feeding and this can persist for 24 - 72 h for some fish (Jobling and Davies 1980). SDA is associated with increased ammonia excretion (Sigholt et al. 1993). Total ammonia nitrogen production and oxygen consumption rate increase on a diurnal pattern (Jarboe 1995, Tanaka and Kadowaki 1995).

As oxygen consumption rates decrease 24 - 72 h after feeding, rates will continue to decrease the longer food is withheld (Jobling 1982, Jobling 1980). Overall nutritional status is reduced because of the lack of complex compounds for catabolism, leading to a reduction in metabolism (Fonds et al. 1992, Jobling 1980).

1.3.2.2 Environmental Factors.

Several environmental factors influence oxygen consumption (Brett 1962), including temperature (Gasca-Leyva et al. 1991, DeMont and O'Dor 1984, Cech et al. 1976, Beamish 1964b), salinity (Gasca-Leyva et al. 1991, Brett 1962), pH (Brett 1962), and hypoxia (Schurman et al. 1991, Kerstens et al. 1979, Cech et al. 1977).

Temperature is a major factor influencing oxygen consumption (Waller 1992, Fivelstad 1988, Brown et al. 1984, Fry 1971). In most poikilotherms temperature is positively related to oxygen consumption rate (Jobling 1994, Jobling 1982, Davis 1975, Beamish 1964b). Acute temperature rises can induce two-fold increases in oxygen consumption rates (Jobling 1982, Voyer and Morrison 1971), while acute temperature declines stimulate the opposite effect (Jobling 1994). Changes in temperature lead to changes in blood-oxygen dissociation curves and affinity of oxygen to haemoglobin (Hayden et al. 1975) which affects total cardiac output (Cech et al. 1976). However, turbot in water warmer than the optimal rearing temperature have decreased oxygen consumption rate (Waller 1992). Whether Northwest Atlantic flatfish have metabolism that is positively related to temperature or metabolism that is related to temperature by a parabolic function needs investigation.

Walleye (*Stizostedion vitreum*) had significantly higher oxygen consumption rates at 25°C than at 20°C (Cai and Summerfelt 1992). Cold water species such as the winter flounder and

Atlantic cod had similar increases in oxygen consumption when the temperature was raised from 5°C to 10°C (Voyer and Morrison 1971, Saunders 1963).

Temperature has no interactive effects with body size in walleye (Cai and Summerfelt 1992). However, weight-specific oxygen consumption of two species of flounder varied with temperature exponentially (Fonds et al. 1992). Temperature directly influences the aerobic metabolism of fish and indirectly influences other factors which result in changes in aerobic metabolism. Therefore, a focus on temperature is important. Intensive culture of fish will either be conducted throughout the annual temperature cycle or be conducted at a temperature which results in greatest growth performance of the fish, depending on the influence of temperature on fish metabolism.

Hypoxia negatively influences oxygen consumption (Schurman et al. 1991, Kerstens et al. 1979, Cech et al. 1977). As water becomes hypoxic, oxygen consumption rate decreases (Cech et al. 1985, Cech et al. 1979) but ventilation rate and cardiac output increase (Kerstens et al. 1979, Cech et al. 1977). In extreme hypoxia, some fish undergo anaerobic metabolism (Cech et al. 1979), while others have a change in behaviour by moving to more optimal local environments (*eg.* within a tank)(Schurman et al. 1991). Chronic exposure to hypoxic environments can lead to reduced development of the gills (McDonald and McMahon 1977), reduced growth (Soderberg 1995), and increased mortality rates (Fry 1971).

Increasing salinity decreases oxygen solubility and decreases oxygen consumption rate (Gasca-Leyva et al. 1991). Changes in pH also lead to changes in oxygen solubility and red blood cell oxygen affinity, affecting gas exchange (Perry and McDonald 1993, Fry 1971). Acidosis reduces the ability of the blood to transport oxygen to the tissues (Wallace 1993). An increase in carbon dioxide (hypercapnia) creates a decreased rbc oxygen affinity and causes increased ventilatory action of the gills (Kinkead and Perry 1991). Interaction of carbon dioxide and pH cause decreased oxygen binding capacity to haemoglobin (Bohr factor) (Cech et al. 1994). Such respiratory challenges may occur under intensive rearing conditions, which emphasizes the importance of proper monitoring systems and understanding the effects and consequences of these stressors.

1.3.2.3 Production factors.

Commercially cultured fish are subject to more intensive conditions than occur in natural populations (Wallace 1993). Even minor changes in behavioral activity (Metcalfe et al. 1995), increased stocking density, enhanced feeding, bacterial growth and pollution influence oxygen consumption and reduce growth (Jobling 1994).

Increasing stocking density positively and negatively influences fish growth, depending on the species, while also inducing some stress responses (Jørgensen et al. 1993, Mazur and Iwama 1993, Kjartansson et al. 1988, Wallace et al. 1988). In chinook salmon (*Oncorhyncus tshawytcha*), increased stocking density of fingerlings above the optimal of 32 kg • m^{-3} has lead to increases in blood cortisol levels and other stress responses (Mazur and Iwama 1993). Increased blood cortisol levels increased oxygen consumption rates (Barton and Schreck 1987) and suppressed growth and disease resistance (Pickering 1993).

Oxygen consumption increased in Arctic charr (*Salvelinus alpinus*) at densities of 15 compared to 60 kg • m^{-3}, but did not increase further at a density of 120 kg • m^{-3} (Jørgensen et al. 1993). Schooling behaviour stimulated a more constant metabolic rate. Rainbow trout supplied with supplemental oxygen had similar oxygen consumption rates at low stocking densities (18 - 30 kg • m^{-3}) and high stocking densities (50 - 70 kg • m^{-3}) (Miller et al. 1995).

Higher stocking densities can induce poor water quality conditions through increases in ammonia and carbon dioxide concentrations and greater accumulation of faecal matter (Wallace 1993). Chronic exposure to high levels of these products negatively affect growth and these waste products compete with fish for available oxygen (Wallace 1993, Rosenthal 1989). In cage culture, waste feed and excretory products can collect on the substrate beneath cages (Rosenthal and Rangeley 1989). High organic loads create deep layers of anoxic sediment (Rosenthal and Rangeley 1989) which consume oxygen and negatively affect the physiology and health of fish in the cage.

Oxygen consumption and aerobic metabolism greatly affect the growth performance of fish by several pathways. The magnitude of their effects can only be determined when oxygen consumption rates are determined under controlled conditions to use as a baseline comparison.

1.4 Objectives.

Many aspects of land-based culture technology which are being considered for flatfish culture in Canada are not clearly defined. However, water flow requirements, as determined by oxygen demands, are critical factors in evaluating the feasibility of production potential, adequate water resources, and site selections. The present study uses single pass, flow-through respirometry to determine the aerobic demands of 3 species of flatfish. The information generated will help assess the influences of several management considerations (species selection, stocking density) on oxygen consumption rates.

The specific objectives of this research are:

1. To compare the routine oxygen consumption of three species of pleuronectids and to determine the degree to which these rates are influenced by water temperatures likely to be encountered during an annual cycle.

2. To determine the influence of increased body mass on the routine oxygen consumption rate of winter flounder in a simulated land-based rearing environment.

3. To compare the influence of increased stocking density on the routine oxygen consumption rate of winter flounder at two temperatures under simulated land-based rearing environments.

2. COMPARISON OF ROUTINE OXYGEN CONSUMPTION RATES OF THREE SPECIES OF PLEURONECTIDS AT THREE TEMPERATURES.

2.1 Introduction.

Finfish aquaculture on the east coast of Canada has developed into a commercially viable industry based mainly on the production of salmonids (Boghen 1995). Strong competition from world producers makes further development in salmonid culture extremely challenging. The diversification of mariculture to new alternate marine species is a logical progression in growth of this industry. Right-eyed flatfish (Pleuronectidae) are among the new marine species being investigated. Several species of this family have been well represented in the commercial fishery (Bowering and Brodie 1991) and have well established market values.

To date, much of the research on new marine species has focused on stages of the early life history of fish such as Atlantic halibut (*Hippoglossus hippoglossus*), wolffish (*Anarhichas* sp), various gaddoids and flounder and on protocols to develop young fish through first feeding and metamorphosis (Brown et al. 1995, Brown and Keough 1994, Brown and Wiseman 1994, Litvak 1994, Waiwood 1994, Holmefjord et al. 1993, Tilseth et al. 1992, Pittman et al. 1990, Tilseth 1990, Waiwood et al. 1988, Blaxter et al. 1983). Future research of new alternate marine species aquaculture should focus on the requirements and potential of both brood fish and production fish.

Water requirements are a major factor limiting an economically feasible land-based aquaculture production site (Fivelstad and Smith 1991, Sigholt et al. 1993). The metabolic requirements of fish in a tank define water requirements (Soderberg 1995). Design of efficient production systems can be influenced by a knowledge of the metabolic requirements of the species.

Although the basic metabolic requirements of many species of flatfish have been well defined (Fonds et al. 1992, Jobling 1982, Wood et al. 1979, Voyer and Morrison 1971) they are poorly understood in terms of aquaculture production, with the exception of turbot, *Scophthalmus maximus*, (Waller 1992, Brown et al. 1984) and Japanese flounder, *Paralichthys olivaceus* (Kikuchi et al. 1990, Honda et al. 1988).

Three species of pleuronectids of potential interest to cold water marine aquaculture in North America are the yellowtail flounder, *Pleuronectes ferruginea*, the winter flounder, *Pleuronectes americanus*, and the American plaice, *Hippoglossoides platessoides*. These three species have traditionally been fished in the wild groundfish industry, though catch numbers have been reduced recently (FAO 1995) and quotas entirely eliminated in some Eastern Canadian commercial fishing zones since 1994 (AGMP 1994).

Respirometry studies focus on one of three states of metabolism. Determining the state of metabolism to be studied is an important factor for evaluation of the significance of the data collected (Cech 1990). 'Standard' metabolism is defined as the amount of energy required to support life by a post-absorptive, non-reproductive fish when no random activity is ongoing (Beamish and Mookherjii 1964). 'Routine' metabolism is the amount of energy required for a fish to survive through a 24 hour cycle (Cech 1990). Studies of routine metabolism involve daily cycles of activity in the form of spontaneous swimming spurts. In general, the fish should remain relatively quiescent or induced to swim no more than 1 bodylength • sec^{-1} (Cech 1990). 'Active' metabolism is evoked from a fish stimulated to its highest sustained swimming speed and is defined as the maximum rate of oxygen consumption for the fish (Fry 1971, Brett 1964). For the purpose of this study, routine oxygen consumption (ROC) is studied because it is the metabolic state most closely associated with land-based aquaculture.

The objective of this study was to compare the ROC rates of three species of pleuronectids at three temperatures using a respirometry technique that simulates land-based aquaculture. Two hypotheses were examined. The first hypothesis was that the ROC rates of the three species were the same under simulated land-based aquaculture conditions. The second hypothesis was that temperature had no influence on the ROC rates of the three species of flatfish.

2.2 Methods.

2.2.1 Fish Collection and Facilities.

All three flatfish species were caught in Passamaquoddy Bay, near St. Andrews, N.B., during May and June, 1995. Fish were caught using an 8 m otter trawl towed for 25 minutes in water depths ranging from 30-70 m. When fish arrived on deck, selected individuals were placed in an insulated plastic tank (0.4 m^3) with flowing seawater (MacIsaac, unpublished). Fish were transported to the Huntsman Marine Science Center, St. Andrews, N.B. and acclimatized to laboratory conditions in 3 m diameter tanks for one month at loading densities less than 5 $kg \cdot m^{-3}$ (MacIsaac, unpublished). Fish were introduced to feed two weeks after being placed in tanks (Goff, unpublished). Their diet included shucked mussel meats and a commercial fish-meal based pellet (Moore-Clarke 3.5 mm dry pellet at 52% protein).

2.2.2 Respirometer Design.

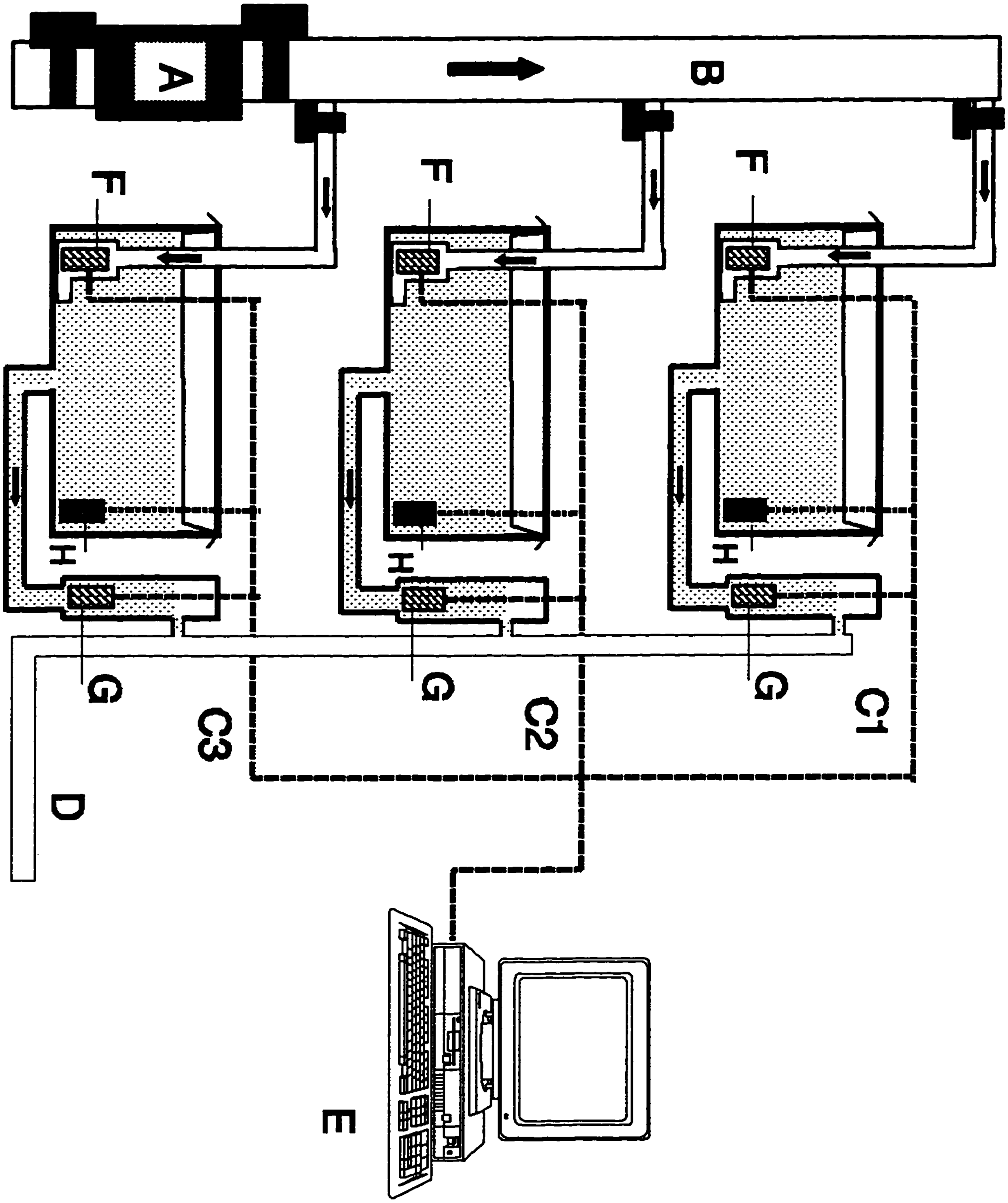
Incoming water was filtered through two 10 μm particle filters and distributed through a 2 m header bar (Fig 1) prior to entering three replicate respirometry chambers. The open-system, single pass, flow-through respirometers, each holding 380 L of water with an average depth of 0.34 m, were used to determine ROC (Fig 2.1). Water entered the tank at the periphery and circulated towards the center drain. A dye test indicated no dead spaces in the chamber. The oxygen probes used to measure dissolved oxygen (DO) were located in the incoming water supply line and in the outflowing standpipe of each respirometer.

Inflow and outflow DO concentrations were measured to $\pm$ 0.01 mg $O_2 \cdot L^{-1}$ using galvanic oxygen probes (*Point Four Systems Inc.*). These measurements were taken continuously and recorded every 4 min (360 times every 24 h) on a computer data logging program (*Oxyguard®*) connected directly to the oxygen probes (Fig 2.1). Flow rates were monitored 4X daily during each experiment. The flow rate for all experiments averaged 3.5 $\pm$ 0.6 L $\cdot$ min^{-1}. During the experiments the outlet DO concentrations did not fall below 6.67 mg $O_2 \cdot L^{-1}$, well above the DO level at which oxygen consumption in flatfish becomes dependent on ambient oxygen supply (Voyer and Morrison 1971). DO readings, flow rate, and tank biomass were used for the calculation of oxygen consumption rate, according to the equation:

$$MO_2 = \frac{[O_2]_{in} - [O_2]_{out} \cdot 60 \text{ min} \cdot h^{-1} \cdot Vw}{W}$$

where MO_2 is the ROC in mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, $[O_2]_{in}$ is the DO concentration in the inflowing water supply, $[O_2]_{out}$ is the DO concentration in the outflowing drain water, *Vw* is the flow rate in L $\cdot$ min^{-1} and W equals the biomass of fish (kg) in the chamber.

During each replication, water temperature varied less than 1°C. The experiment was housed in an isolated room, receiving daylight in addition to a 40 watt lightbulb synchronized to the natural photoperiod.


A
B
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G
C1
C2
C3
D
E

Fig. 2.1 Diagram of the respirometers. I) Water is supplied from the main pipeline and passes through two 10 μm particle filters (a). Water then flows into a 2 m header bar (b) from which it is distributed to three replicate respirometers through inflowing lines, circulates through the chambers (c1, c2, c3) and exits by the drain (d). All data is sent to the computer hardware (e). II) Initial oxygen concentration is measured at the inflowing oxygen probe (f), and outflowing oxygen concentration is measured with the outflowing oxygen probe (g). Temperature is monitored with temperature thermistors (h).

2.2.3 Experimental Protocol.

The sizes of the fish used are described in Table 2.1. The stocking density for these experiments approximated 3 kg • m^{-3} or 1 kg • m^{-2}.

Before each experiment all DO probes were calibrated at a constant temperature to 0% saturation in water using sodium sulfite and then to 100% saturation in an aerated water bath. Before each trial a baseline equilibrium was initiated in each respirometer. A baseline equilibrium was defined after the readings of the outlet probes were corrected to match the readings of the inlet probes and the two probes recorded stable, similar readings for at least 2 h.

Flatfish had been placed into staging tanks identical to the experimental respirometers, for 48 h, to allow the fish to acclimate and to provide time for the fish to empty their gastrointestinal tract. This follows the recommendations of Jobling (1980) for respirometry studies.

Fish were placed into the respirometry chambers following the baseline measurement and given an additional 12-16 h (from early evening through mid morning) for acclimation before measurements began. ROC data were measured for 24 h following the acclimation period in the respirometer. Fish were not fed during the trial period. This provided some degree of control of bacterial oxygen consumption because no organic substrates (*eg.* waste feed, faecal matter) were available for bacterial cultures (Cech 1990, Dalla Via 1983).

2.2.4 Replication of Trials and Statistical Methods.

Each of the three species was allocated to one of the three respirometers. The experiment was replicated three times according to a three by three Latin Square experimental design such that each group of fish was tested in each of the three respirometer chambers (Steel and Torrie 1980).

TABLE 2.1.

Size of the experimental animals used in the respirometry study at all 3 temperatures.

Species	Weight Range (g)	Avg. Density ($kg \cdot m^{-3}$)	Avg. Fish Mass (g)	Number of fish
American plaice	50 - 370	3	166	4 - 11
winter flounder	60 - 240	3	150	4 - 12
yellowtail flounder	52 - 410	3	176	2 - 11

Using this procedure, the differences in the ROC rate of each species at one temperature, and the differences of any one species across the three temperatures, could be determined while accounting for tank effects.

A limited number of oxygen values in the replications were influenced during daily operating and maintenance procedures (eg. during daily cleaning of filters). Oxygen values recorded during disturbance periods of any respirometer were deleted and matched recordings from the other two respirometers were systematically deleted to eliminate that influence on the analysis. Our number of observations used in the analysis is smaller than the maximum possible number produced by the data logging program.

The hypotheses were tested by a 3-way ANOVA in SAS version 6.02. Three factors were analyzed: species, temperature, and trial, with a tank factor nested in trial. The significance was based on an α equal to 0.05. Where factors indicated significant effects on the outcome, Student Newman-Kuels (SNK) non-parametric tests were performed to determine if the levels in the factor were significantly different from each other.

2.3 Results.

All ROC rates (mean ± s.d.) at each of the temperatures tested are summarized in Table 2.2. The first step of the analysis tested any interactions among the factors involved. Each of the interactions among the three factors had a significant effect on the outcome (Table 2.3). Due to the significant interaction effects, the dataset collected at each temperature tested was analyzed individually using a one-way ANOVA. When temperature was held constant and the effect of species was tested, two factors were involved: species and trial, with a tank factor nested in trial.

At the coldest water temperature (2°C) the measured ROC rates differed significantly ($P<0.05$) among species. A SNK non-parametric test of the means indicated each species had a significantly different mean oxygen consumption rate ($P<0.05$, Table 2.2). The American plaice had the highest oxygen consumption rate, and the yellowtail flounder had the lowest. In a separate holding tank which contained all 3 species, the American plaice and the yellowtail flounder had distended stomachs at this temperature, an indication of continued feeding at the cold temperature. Winter flounder in the same tank had concave shaped stomachs, an indication of little feeding.

At 11°C, ROC rates also varied significantly ($P<0.05$) among species. A SNK testof the means indicated that all species had significantly different ROC rates ($P<0.05$, Table 2.2). The yellowtail flounder had the highest oxygen consumption rate, and the American plaice had the lowest.

At the warmest water temperature (14°C) ROC rate again varied significantly among species ($P<0.05$). A SNK test of the means indicated that all species had significantly different ROC rates ($P<0.05$, Table 2.2). The American plaice had the highest ROC rate and the yellowtail flounder had the lowest.

A second series of one-way ANOVA tests was conducted for the species to determine the effect of temperature on each species' ROC rate. Two factors used in the ANOVA were temperature and trial, with the tank factor nested in trial.

Temperature had a significant effect on ROC rate in American plaice ($P<0.05$). The highest ROC rates measured for the American plaice were at 14°C, the lowest ROC measured at 11°C (Table 2.2). A SNK test of the mean ROC rates for the American plaice indicated that ROC rates were significantly higher at 14°C compared to the other two temperatures ($P<0.05$).

TABLE 2.2.

Mean (± s.d.) routine oxygen consumption rate (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) for the American plaice, winter flounder, and yellowtail flounder repeated at 3 temperatures in respirometers in a simulated aquaculture tank environment. Values in columns with dissimilar superscripts are different at $P<0.05$ (according to a SNK test).

Species	Temperature		
	2°C	11°C	14°C
American plaice	73 ± 25^a	67 ± 45^a	129 ± 62^a
winter flounder	63 ± 30^b	220 ± 70^b	118 ± 41^b
yellowtail flounder	40 ± 19^c	292 ± 85^c	91 ± 19^c
Sample Sizes (N)	479	546	444

TABLE 2.3.

Statistical evaluation of the interaction of species, temperature and trial in determining differences in ROC among 3 species of pleuronectids tested at 3 temperatures.

Factor	Df	F	Probability
Temperature x Species	4	1490.51	0.0001
Species x Trial	4	5.48	0.0193

Temperature had significant effects on the ROC rates of both the yellowtail flounder and winter flounder ($P < 0.05$). For both species ROC increased as temperature increased from 2°C to 11°C, and decreased as temperature increased from 11°C to 14°C (Table 2.2). SNK tests of the mean ROC rates of both flounders at each temperature indicated significant differences from each other ($P < 0.05$).

No evidence of bacterial oxygen consumption within the respirometers was observed as indicated by stable oxygen consumption values throughout the measurement period.

2.4 Discussion.

Temperature directly affected the ROC of the flounders studied. In 2 of the 3 species of flounder ROC rates followed a parabolic-like curve similar to ROC rates of turbot (Waller 1992). Waller (1992) suggests this response in turbot reflected the optimal temperature range in turbot. Imsland et al. (1995) determined increasing oxygen consumption rates as the temperatures approached the optimal rearing temperature in turbot. The limited number of temperatures tested in this study does not enable the true optimal rearing temperature range to be isolated, but the temperature range is apparently within 2 - 14°C, and the optimal temperature is likely at 11°C.

As with all poikilothermic fish, metabolic rates decrease as temperatures decrease (Hazel 1993). Fish have lower metabolism and a lower degree of activity at colder temperatures (Fonds et al. 1992, Jobling 1982). Increase in temperature generally leads to increase in oxygen consumption and other physiological rates including increased growth rate (Fonds et al. 1992) over optimal temperature ranges.

At the cold temperature, American plaice had the highest rate of ROC. These flounder had large, distended stomachs while in holding tanks during the colder temperatures. Distended stomachs may reflect increased feeding, greater activity or slower passage through the gastrointestinal tract at colder temperatures. Scott and Scott (1988) reported American plaice have a northern distribution in optimal water temperatures from just below zero to 1.5°C. Frank et al. (1992) indicated that cold water temperatures from the Labrador Current influence the spawning pattern of this cold water species. This species adapted to cold water temperatures and is closest to its optimal temperature at the low temperature tested in this study.

The yellowtail flounder was associated with warmer water temperature and was near the northern limit of its distribution on the Grand Banks (Frank et al. 1992, Bowering and Brodie 1991). Scott and Scott (1988) reported the optimal temperature range as 2°-12.2°C. Bigelow and Schroeder (1953) reported the distribution of the yellowtail from north of the Gulf of Maine south to Cape Cod in water temperatures from 0°-14°C. Similar distributions and temperature ranges have been described for the winter flounder (Scott and Scott 1988, McCracken 1963). During the period of cold water experiments, the winter flounder in holding tanks displayed minimal or no feeding or other activity and animals had concave-shaped stomachs. Hoornbeek et al. (1982) reported that during a growth trial, winter flounder had decreased feeding activity below 5°C. Growth rates of these fish were less than those maintained in warm (15°C) water temperatures (Hoornbeek et al. 1982), suggesting marked differences in metabolism at the two temperatures.

The increased ROC of two species at 11°C indicated that these animals had higher metabolism at this temperature. Scott (1954) reported periods of rapid growth on scale and otolith samples of yellowtail flounder during late spring and summer from the Middle Ground and Western Bank. He also observed similarities in patterns of otolith and scale growth of yellowtail flounder and winter flounder. These similarities in temperature and growth may have been reflected in metabolic rates determined herein.

At 14°C, the ROC rates of the yellowtail and winter flounders were reduced. As these two species approach the upper limit of their temperature range their physiological response in metabolism is similar to the oxygen consumption rates in turbot which demonstrate a parabolic response to temperature (Waller 1992). The increase in oxygen consumption by the American plaice is more difficult to explain and merits further examination.

Most available literature on standard metabolism was data obtained under controlled conditions where individual fish were monitored and therefore the influences of stocking density and behaviourial interactions were not considered. Data from standard metabolic studies underestimate oxygen consumption in production systems. Alternatively, routine metabolism provide more relevant data for extrapolation to production level systems. Benthic fish of the family Pleuronectidae are stationary for long periods on a substrate (Scott and Scott 1988, Gibson 1975, Bigelow and Schroeder 1953). Hence, studying active metabolism was difficult, requiring a tilting respirometer for flatfish similar to that of Priede and Holliday (1980). Based on the observations of fish activity in our respirometers, the oxygen consumption rates in the present study are routine metabolism as defined by Cech (1990) and Fry (1971). Using routine metabolic studies with groups of fish is a realistic estimate of production requirements of these species in land-based aquaculture.

The ROC rates collected for these species were compared with the consumption rates reported for other flatfish species and selected pelagic species in Table 2.4. ROC rates were higher than those estimated for standard metabolism of winter flounder and starry flounder (Wood et al. 1979, Voyer and Morrison 1971) and were collected across a wider range of temperatures. Measuring oxygen consumption under culture conditions elicited higher rates than under laboratory conditions for turbot (Brown et al. 1984). The ROC rates of this study were more similar to ROC values of Atlantic halibut (Davenport et al. 1990) although that study was conducted with larger sized fish and only at 10°C. The critical comparison is how much less the values from this study on flatfish were compared to salmonids (Fivelstad and Smith 1991, Webb 1971, Beamish 1964c). The maximum ROC in all flatfish species in this study was less than the lowest oxygen consumption rate of Atlantic salmon (*Salmo salar*) under similar holding conditions (Fivelstad and Smith 1991). Average ROC rates for flounder at comparable temperatures approximate those of salmonids under standard metabolic conditions, and were much less than salmonids under active metabolic conditions (Table 2.4) (Webb 1971, Beamish 1964c).

An increasing amount of variability was measured at the two higher temperatures tested. This was not surprising for the yellowtail and winter flounders because of the increased amount of activity by the animals in warmer water. Routine activity spans the wide range between standard and active metabolism, so the variation in activity of the fish during the experimental period was an inherent source of error in this type of respirometry technique.

Other variation in the data collected for these species was likely due to environmental influences over which there was little experimental control. Intertidal species, such as the winter flounder, have shown increased movement into intertidal zones as the tides change (Gibson 1975, Tyler 1971). Water pumped to the facilities came from just below the intertidal zone, which fluctuates over 8 meters of amplitude. Though responses by habituated fish were not compared with tidal fluxes, perhaps the behaviour of these fish during changes in tides (*eg*. environmental

TABLE 2.4.

Oxygen consumption rates of flatfish and salmonid species.

Species	Activity	Rate**	Temp.	Size (g)	Source
American plaice	Routine	67-129	2-14°C	50-370	present study
winter flounder	Routine	63-219	2-14°C	60-240	present study
yellowtail flounder	Routine	39-292	2-14°C	52-410	present study
winter flounder	Standard	55	10°C	14-32	Voyer and Morrison 1971
winter flounder	Standard	97	20°C	14-32	Voyer and Morrison 1971
turbot	Routine	32-227	8-24°C	1.5-1073	Waller 1992
Atlantic halibut	Routine	50-80	10°C	454-2334	Davenport et al. 1990
Atlantic salmon	Routine	200-500	5-9°C	200-850	Fivelstad and Smith 1991
rainbow trout	Standard	74-76	15°C	258-291	Webb 1971
rainbow trout	Active	660	15°C	258-291	Webb 1971
brook trout	Standard	77	10°C	100-275	Beamish 1964c

** (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$)

cues, hormonal cues) would influence the variation measured in the experiments.

The winter flounder may be more suited to environments where winter temperatures are higher. The yellowtail flounder may be a promising aquaculture candidate in cold environments that warm up moderately throughout the summer months. Both species showed increased ROC with increasing temperature up to 11°C. Additional trials could refine a species-specific temperature associated with peak metabolism. A strong correlation with this pattern will likely emerge when temperature specific growth rates of these species are measured. Bejda et al. (1992) indicated strong correlations between growth rates of winter flounder and levels of dissolved oxygen, supporting the value of oxygen consumption studies in determining suitable rearing protocols of flatfish. The culture of American plaice may be best restricted to areas which are subjected to colder water temperatures.

The single pass, flow-through respirometry technique developed in this study served as a valuable tool which answers many questions on the demands of species in land-based aquaculture. Comparisons among species can be identified and quantified under standard conditions. Appropriate estimates of the demands of groups of fish are possible, as is required in an aquaculture setting (Brown et al. 1984). The present respirometry technique is a useful tool to monitor the aerobic demands of flatfish under intensive holding conditions. The results in this study are the most appropriate estimate of the oxygen requirements of flatfish for aquaculture. Obtaining these data is a critical prerequisite to evaluating other conditions including the oxygen consumption of flounders during feeding and at increased stocking densities.

3. OXYGEN METABOLISM OF WINTER FLOUNDER (*PLEURONECTES AMERICANUS*) IN AQUACULTURE TANKS - THE INFLUENCE OF FISH SIZE AT THREE TEMPERATURES.

3.1 Introduction.

Oxygen is a limiting factor in aquaculture production, as are accumulations of ammonia and carbon dioxide (Tanaka and Kadowaki 1995, Brown et al. 1984). Knowledge of the oxygen requirements of cultured species permits selection of appropriate water flow rates (Fivelstad 1988) which will ultimately affect the economic feasibilty of land-based aquaculture operations (Fivelstad and Smith 1991, Sigholt et al. 1993). Somatic factors, such as fish size, influence oxygen metabolism for various vertebrate species, including flatfish species such as turbot, *Scophthalmus maximus*, and European plaice, *Pleuronectes platessa*, (Fonds et al. 1992, Waller 1992, Brown et al. 1984, Jobling 1982). Changes in metabolism associated with ontogenetic development of a species (Kikuchi et al. 1990) are important to determine for aquaculture production.

The effect of environmental factors, such as water temperature, on oxygen metabolism have also been documented for certain flatfish including turbot, plaice, and winter flounder, *Pleuronectes americanus*, (Fonds et al. 1992, Waller 1992, Brown et al. 1984, Voyer and Morrison 1971). Seasonal temperature differences influence responses in poikilothermic fish and consequently water flow requirements for rearing must be estimated throughout the annual temperature cycle. For example, turbot have a parabolic response to temperature with a maximum oxygen consumption between 16 - 19°C (Waller 1992). European plaice and winter flounder double their oxygen consumption rate in response to acute increases in water temperature (Jobling

1982, Voyer and Morrison 1971). The responses in ROC of winter flounder to gradual changes in temperature is not clear.

Winter flounder is a cold water species of interest as a potential candidate for aquaculture. The oxygen consumption requirements of these fish are not clearly defined, though some data are available in studies on larval (Laurence 1975) and pre-juvenile stages (Voyer and Morrison 1971). Similar studies on turbot (Brown et al. 1984) and Japanese flounder, *Paralichthys olivaceus*, (Kikuchi et al. 1990) indicate that fish size influences oxygen consumption under culture conditions. The present study was conducted using single-pass, flow-through respirometry in an aquaculture tank environment to determine the effect of fish size on the ROC of winter flounder. The experimental fish included a range of sizes that are encountered during the production phase. The experiment was repeated at 3 temperatures to determine the influence of temperature and to measure interactions between the effect of fish size and the influence of temperature on oxygen consumption.

3.2 Methods.

At least one month prior to each experiment, winter flounder were collected from the wild as described in Chapter 2. Fish were collected in spring 1995 from 5°C seawater, in fall 1995 from 6°C seawater, and in spring 1996 from 4°C seawater. The sizes of the experimental fish used during the three experiments are shown in Table 3.1. In each experiment, the stocking density was 5 kg • m^{-3}.

During the habituation period for the flounder, temperature changed less than ± 0.5°C • day^{-1}. The first experiment was conducted during summer 1995 at 13°C ± 0.3°C, the second was

TABLE 3.1

Sizes of the winter flounder used in the respirometry experiments.

Temp. (°C)	Size Group	No. of fish	Total Mass (kg)	Average Mass (g) ± s.d.
2°C	Small	10	1.77	177 ± 12.5
	Mid	7	1.76	251 ± 21.9
	Large	4	1.75	438 ± 15.0
9°C	Small	19	1.79	94 ± 16.0
	Mid	9	1.71	190 ± 11.2
	Large	5	1.75	350 ± 29.2
13°C	Small	24	1.79	75 ± 18.2
	Mid	8	1.84	230 ± 22.7
	Large	4	1.82	455 ± 125.0

conducted during winter 1996 at 2°C ±0.1°C and the third was conducted during spring 1996 at 9 ± 0.3°C. Water flow rates were measured 4X during each trial using a 3 L beaker and a timer and averaged 3.7 ± 0.4 $L \bullet min^{-1}$.

Routine oxygen consumption data were collected using single-pass, flow-through respirometry as described in Chapter 2. Rates of routine oxygen consumption were determined based on measurements of the difference in oxygen concentrations between each respirometer's inlet supply water and effluent drain water, water flow rates, and the biomass of fish in the tank. This oxygen consumption was expressed in units of $mg\ O_2 \bullet kg^{-1} \bullet h^{-1}$.

The experiment was repeated at each temperature using to a 3 by 3 Latin Square design such that each size group of flounder was tested in each of the three respirometers (Steel and Torrie 1980). Disturbances such as flow interruptions during daily maintenance procedures occurred during the measurement periods. Data collected during these periods was censored from the datasets. Least squares regression (Cody and Smith 1991) was used to determine the relationship between fish size and oxygen consumption, based on a significance level of $\alpha > 0.05$.

3.3 Results.

The oxygen consumption rates of the size groups of winter flounder are listed in Table 3.2. The groups of smaller flounder consumed more oxygen than the groups of larger flounder. Linear regression equations describing the relationship of oxygen consumption to fish size are listed in Table 3.3. Each equation had a slope significantly less than 0 ($P<0.05$). The rate of oxygen consumption per unit of body weight decreased with increasing fish size. However the Pearson correlation coefficient of determination (r^2) for each of the equations was low (0.14 at 2°C, 0.6

at 9°C and 0.45 at 13°C).

To improve the fit of the model to the data, oxygen consumption rates per unit of body weight were manipulated into oxygen consumption rates per fish (mg $O_2 \cdot fish^{-1} \cdot h^{-1}$). Logarithmic transformations of oxygen consumption per fish and fish size were done followed by regression analysis. Zeuthan (1953) stated the relationship between oxygen consumption and body size was exponential according to the equation $MO_2 = aW^b$, where MO_2 is mg $O_2 \cdot fish^{-1} \cdot h^{-1}$, W is mass (grams), 'b' is the slope which describes the function. The 'a' is the y-intercept which is temperature dependent. This procedure enabled the data to be compared to previously published data for the effect of fish size on oxygen consumption.

The equations describing the relationship between oxygen consumption per fish and fish size are in Table 3.4. The estimated slopes of the equations are 0.66 at 2°C, 0.19 at 9°C, and 0.4 at 13°C. However, the procedure of log transformation of the data did not improve the fit of the model to the data. The r^2 values were lower than the r^2 values for the standardized estimates (0.05 at 2°C, 0.01 at 9°C, and 0.12 at 13°C). These regression estimates are presented only for comparison with previous reports.

According to a three way analysis of variance, temperature and fish size significantly interacted with oxygen consumption per unit of body weight ($P<0.05$). In addition, each estimate of 'a' was significantly different from each other ($P<0.05$). Oxygen consumption rates were significantly higher at 9°C than at the other two temperatures ($P<0.05$), but were not significantly different at 2°C than at 13°C ($P>0.05$).

When trial and tank were added to the regression model as dummy variables, a general equation which explained the relationship between oxygen consumption, fish size, temperature,

TABLE 3.2

Oxygen consumption rates (mg O_2 • kg^{-1} • h^{-1}) of groups of winter flounder at varying sizes measured at 3 temperatures.

Temp (°C)	Mean weight (g) ± s.d.	O_2 Consumption (mg O_2 • kg^{-1} • h^{-1}) ± s.e.m.
2°C	177 ± 12.5	54.1 ± 1.8
	251 ± 21.9	61.5 ± 1.4
	438 ± 15.0	38.5 ± 1.3
9°C	94 ± 16.0	131.5 ± 1.3
	190 ± 11.2	78.7 ± 1.9
	350 ± 29.2	73.9 ± 2.4
13°C	75 ± 18.2	83.6 ± 1.3
	230 ± 22.7	65.0 ± 1.5
	455 ± 125.0	35.2 ± 1.1

TABLE 3.3

Least squares regression of oxygen consumption rates (MO_2: mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and body weight (g) at temperatures of 2°, 9°, and 13°C. W is mean fish size (g), T is temperature (°C), R is trial and C is tank.

Temp. (°C)	Weight Range (g)	Linear Regression	r^2
2°C	177 - 438	$MO_2 = 95.0 - 0.08(W)$	0.14
9°C	94 - 350	$MO_2 = 205.7 - 0.2(W)$	0.60
13°C	75 - 455	$MO_2 = 41.9 - 0.13(W)$	0.45

Whole model: $MO_2 = 118.9 + 1.05(T) - 0.16(W) + 14.3(R) - 22.1(C)$ 0.32

TABLE 3.4

Least squares regression of the log of oxygen consumption per fish (MO_2: mg O_2 • $fish^{-1}$ •h^{-1}) and the log of body weight (W: g) at temperatures of 2°, 9°, and 13°C.

Temp. (°C)	Weight Range (g)	Linear Regression	r^2
2°C	177 - 438	$MO_2 = -0.7\ W^{0.66}$	0.05
9°C	94 - 350	$MO_2 = 0.65\ W^{0.19}$	0.01
13°C	75 - 455	$MO_2 = 0.03\ W^{0.4}$	0.12

trial and tank was determined. In this model, all factors were significantly different from 0 ($P<0.05$). The model which explains the data is

$$MO_2 = 118.9 + 1.05T - 0.16W + 14.3R - 22.1C$$

where $MO_2 = \text{mg } O_2 \bullet \text{kg}^{-1} \bullet \text{h}^{-1}$, W is mean fish size (g), T is temperature (°C), R is trial and C is tank. Using 10°C and a 200 g flounder as an example, the oxygen consumption rate would be 89.6 $\text{mg } O_2 \bullet \text{kg}^{-1} \bullet \text{h}^{-1}$.

3.4 Discussion.

The oxygen consumption rates determined in this study for flounder are defined as the ROC rates of food deprived flounder. Flounder regularly received daily rations of pelleted feed while in holding tanks until the experiments were conducted. Flounder were offered food until satiation during each daily feeding rather than a prescribed percentage of the total biomass of the tank. This ensured that all flounder took food. Before oxygen consumption rates were measured, food was withheld for 48 h to reduce faecal production while in the respirometer to avoid high levels of bacterial oxygen consumption (BOC) (Dalla Via 1983, Jobling 1980). Jobling and Davies (1980) report the duration of specific dynamic action (SDA) is temperature and ration size dependent, being longer than 50 h at 10°C and longer than 35 h at 15°C before oxygen consumption returned to pre-feeding levels. Using Jobling's estimates for SDA and assuming ration sizes were below maximum, flounder in the respirometers at 9°C and 13°C were at or below pre-feeding levels through most of the data collection period, and thus should have lower oxygen consumption rates. In European plaice, oxygen consumption rates gradually declined the longer food was deprived. Jobling (1982) suggested that during an extended measurement period, such as 24 hours, oxygen consumption levels may have decreased well under pre-feeding levels.

When water temperatures declined below 5°C, winter flounder went off feed and regular feedings did not resume until spring. Flounders used in the low water temperature experiment (2°C) had been maintained on reserve energy for several weeks.

In this study low ROC rates were determined based on the feeding status of the fish (Jobling 1982) and the flow-through respirometry technique (Cech 1990). Fish were fed before experiments and between replications, but the acclimation and data collection times were adequate to ensure oxygen consumption rates were of fasted fish. Therefore, the oxygen consumption rates determined in this study, across all temperatures, are the minimum aerobic requirements for flounder in tanks.

The results of this study indicate that oxygen consumption rates of winter flounder decrease as fish size increases. The r^2 values for each of the regression estimates were very low. However the flounder used in the study were captured from the wild and the groups were not uniform in size. This variability in the independent variable would contribute to the low r^2. The data collection period was conducted over 24 hours on a natural photoperiod. Imsland et al. (1995) reported that turbot had lower oxygen consumption rates during dark periods compared to light periods. The increased variability resulting from measuring oxygen consumption over a light and dark photoperiod would also contribute to a lower r^2. Tyler (1971) found that winter flounder in the Passamaquoddy Bay, N.B., moved into the intertidal zone to feed during high tides. Any endogenous responses by the winter flounder associated with the changing tides would increase the variance in the oxygen consumption rates and contribute to a lower r^2.

Linear regression of oxygen consumption per unit of body weight against average body size gave better model estimates than logarithmic transformation of oxygen consumption per fish and body size. This is in contrast to published literature addressing the effect of body size on oxygen consumption in flatfish (Waller 1992, Jobling 1982), other freshwater and saltwater fish species including eel, *Anguilla anguilla* (Degani et al. 1989), tilapia, *Tilapia mossambica* (Job 1969), brown trout, *Salmo trutta*, brook trout, *Salvelinus fontinalis*, common white sucker, *Catostomus commersonii*, brown bullhead, *Ictalurus nebulosus*, and carp, *Cyprinus carpio*, (Beamish 1964b), and aquatic organisms in general (Zeuthan 1953). The above studies consistently described the relationship of oxygen consumption (mg $O_2 \bullet fish^{-1} \bullet h^{-1}$) according to an exponential function with a weight exponent varying from 0.6 to 0.9 (Jobling 1994).

The estimates of the weight exponent in winter flounder from the present study were less than values published previously, except for the cold water experiment (2°C) which had an exponent of 0.66. However, the sizes of flounder used spanned the range from 1.5 - 1000 g (Waller 1992). The extent of this range is conducive to measuring an exponential relationship associated with early juvenile growth. When the range is reduced, the relationship could be predicted according to a different model. For example, a third degree polynomial was used to describe the relationship between body size and oxygen consumption in winter flounder of various stages of larval growth and metamorphosis (Laurence 1975). Jobling (1982) used an exponential function to describe the relationship, but in a sample group ranging from 12 - 20 g, the weight exponent was 1.47. Over small intervals of body size, such as Laurence (1975) and the present study, exponential relationships may not be the most appropriate model to predict oxygen consumption rates.

The linear model which described my data is more appropriate than other models because of the range of sizes used herein. The sizes of winter flounder in the present study represents a group undergoing little ontogenetic change. Kikuchi et al. (1990) determined that oxygen consumption rates of Japanese flounder over a size range of 10.8 - 878 g were influenced by body size according to the equation $MO_2 = 48.9\ W^{-0.45}$ where MO_2 was mL $O_2 \bullet 100\ g^{-1} \bullet h^{-1}$ and W was body size (g). Over this size range the rate of change in oxygen consumption was more linear than exponential.

Studying a larger size range of fish would have higher probability of displaying an exponential relationship between body size and oxygen consumption rate. However, the objective of this study was to determine this relationship between flounder of sizes encountered during the grow-out phase of production. The model which has been presented describes this relationship and enables flow regimes to be determined for land-based aquaculture operations holding these size flounder.

For example, a tank containing 190 g flounder is held at 9°C. According to the results of this study, flounder of this size and at this temperature would have a minimum oxygen consumption rate of 79 mg $O_2 \bullet kg^{-1} \bullet h^{-1}$. Based on the guidelines of Brown et al. (1984) a rearing tank containing a volume of 33 m^3 and at a stocking density of 40 kg $\bullet\ m^{-3}$ of flounder would be practical (7000 flounder, average size 190 grams). Supply water, at a 100% oxygen saturation, would contain 9.5 mg $O_2 \bullet L^{-1}$. Growth in winter flounder is affected by level of dissolved oxygen (Bejda et al. 1992), so maintaining oxygen saturation above 90% in the tank is an ideal objective. Using these parameters, a minimum flow rate of 185 L $\bullet\ min^{-1}$ would be required to safely provide adequate dissolved oxygen. Oxygen consumption rate is elevated during feeding periods (Brett and Zala 1975). In plaice, oxygen consumption increases as much as much as twice the resting level during feeding (Jobling and Davies 1980). This would require a flow rate of at least 370 L • min^{-1} in the tank to maintain a constant 90% saturation in the tank. Alternatively a temporary reduction (*eg*. 20 min) in dissolved oxygen saturation immediately following feeding may be acceptable. Future studies will determine the oxygen requirements of various sized flounder during feeding.

The objective of this study was to determine the effect of fish size on the oxygen consumption rates of winter flounder using a modified respirometer which simulated the holding conditions of intensive land-based aquaculture. The relationship of body size to oxygen metabolism at 3 temperatures was studied. The temperature related changes in metabolism generally resemble the response of turbot to a wide temperature range (Waller 1992).

The model developed best describes the data collected using this technique. Repeating this study when cultured winter flounder of identical sizes are available will improve the prediction between fish size, temperature and oxygen consumption.

4.0 THE INFLUENCE OF STOCKING DENSITY ON THE ROUTINE OXYGEN CONSUMPTION OF WINTER FLOUNDER AT TWO TEMPERATURES.

4.1 Introduction.

Stocking density influences growth (Björnsson 1994, Jørgensen et al. 1993, Wallace et al. 1988, Papoutsoglou et al. 1987), survival (Holm et al. 1990, Refstie 1977), stress (Mazur and Iwama 1993), behaviour (Brown et al. 1992), and oxygen consumption (Björnsson 1994, Jørgensen et al. 1993, Honda et al. 1988). Determining the oxygen consumption of flounder is a prerequisite in the evaluation of flatfish culture potential. Stocking density and water flow requirements are two components limiting the feasibility of land-based aquaculture (Kjartansson et al. 1988) which are predicted according to oxygen consumption rates (Honda et al. 1988, Brown et al. 1984). Chronically high stocking densities induce crowding stress eliciting secondary stress responses such as metabolic disturbances (Kjartansson et al. 1988) which negatively affect growth (Refstie 1977). Low flow rates can lead to chronically hypoxic environments which result in reduced growth rates in winter flounder, *Pleuronectes americanus* (Bejda et al. 1992).

The oxygen consumption rates of Japanese flounder, *Paralichthys olivaceus*, (Honda et al. 1988), Atlantic halibut, *Hippoglossus hippoglossus* (Björnsson 1994) and turbot, *Scaphthalmus maximus*, (Brown et al. 1984) at high densities are useful in establishing an upper limit to the rearing capacity of those flatfish in tanks. Determining optimal and upper limit stocking densities based on ROC rates of flounder is important because over-loading production tanks with fish could suppress growth and increase mortality rates (Miller et al. 1995, Martinez-Tapia and Fernández-Pato 1991). The objective of this study was to determine the influence of stocking density on routine oxygen demands of winter flounder.

4.2 Methods

Winter flounder were collected in late fall 1995 from 6°C seawater and spring 1996 from 4°C seawater in the same manner as in Chapter 2. During the habituation of the flounder to the laboratory temperature changed less than 0.5°C • day^{-1}. The experiment was repeated at two temperatures. The first experiment was conducted during winter at 2.2°C ± 0.3°C. The second experiment was conducted during the early summer at 9.7°C ± 0.3°C.

The sizes of the fish used in the experiments are listed in Table 4.1 and stocking densities of 5 kg •m^{-3}, 25 kg • m^{-3}, and 50 kg • m^{-3} were used. Oxygen is available in the water in three dimensions so the stocking densities reported throughout this study have the units of kg • m^{-3}. For comparison, equivalent stocking densities are also listed in Table 4.1 in units of kg • m^{-2}. Fish were weighed individually at the beginning of each temperature experiment. Because fish were supplied from the wild some variability occurred in flounder size. The sizes of flounder in each density group cover a range of sizes which would be used in ongrowing aquaculture operations. Including larger fish in each density group allowed the test stocking density to be attained, although the influence of body size on oxygen consumption per unit of body wieght is an important factor to control (Waller 1992, Jobling 1982). However, each density group was set up consisting of equal proportions of each size of flounder (Table 4.2). For this study larger flounder were added to attain experimental stocking densities which ensured the data represented the effect under investigation.

TABLE 4.1

Sizes of the winter flounder used to study the influence of stocking density on the oxygen consumption of winter flounder.

Temperature (°C)	Density ($kg \cdot m^{-3}$)	($kg \cdot m^{-2}$)	Estimated % Coverage of tank.	Avg. mass (± s.d.) (g)	Range Fish Mass (g)
2.2°C	5	2	13	354 ± 100	270 - 520
	25	8	67	373 ± 93	220 - 560
	50	16	130	373 ± 120	220 - 660
9.7°C	5	2	15	325 ± 153	190 - 620
	25	8	67	276 ± 72	170 - 560
	50	16	130	320 ± 120	180 - 780

TABLE 4.2

Size composition of flounder in the three density groups. Proportions are equal to the weights of the fish multiplied by the number fish in that weight range divided by total biomass of the tank (number of fish in brackets).

9.7°C	5 kg • m^{-3}	25 kg • m^{-3}	50 kg • m^{-3}
under 200 g	9% (1)	6% (3)	6% (5)
200-250 g	12% (1)	29% (11)	17% (14)
250-300 g	30% (2)	22% (7)	22% (14)
300-350 g	17% (1)	33% (9)	9% (5)
350-400 g	-	4% (1)	17% (8)
400-450 g	-	-	10% (4)
450-500 g	-	-	8% (3)
500-550 g	-	-	3% (1)
550-600 g	-	6% (1)	-
600-650 g	32% (1)	-	-
650g +	-	-	8% (2)

2.2°C	5 kg • m^{-3}	25 kg • m^{-3}	50 kg • m^{-3}
under 200 g	-	-	-
200-250 g	-	7% (3)	7% (5)
250-300 g	31% (2)	3% (1)	19% (12)
300-350 g	19% (1)	29% (8)	13% (7)
350-400 g	20% (1)	13% (3)	19% (9)
400-450 g	-	15% (3)	15% (6)
450-500 g	-	27% (5)	6% (2)
500-550 g	29% (1)	-	-
550-600 g	-	6% (1)	3% (1)
600-650 g	-	-	11% (3)
650g +	-	-	4% (1)

A sample of 8 flounder was weighed and their surface area (cm^2) was estimated. The data points were then used to estimate the relationship between surface area (SA: cm^2) and body mass (W: g) which was described as

$$SA = 0.607\ cm^2 \cdot g^{-1}(W) + 87.21\ cm^2.$$

The surface area of the respirometer tanks was 11310 cm^2. Using the equation for surface area for flounder at the three stocking densities and dividing by the surface area of the respirometer, % coverage of the tank was estimated. The upper densities were similar to experimental densities tested for Atlantic halibut (Björnsson 1994), turbot (Martinez-Tapio and Fernández-Pato 1991, Brown et al. 1984), and Japanese flounder (Honda et al. 1988). The lower densities were similar to experimental densities used in related experiments.

Routine oxygen consumption rates were determined using single-pass flow-through respirometry (see Chapter 2). A condition maintained throughout each trial was that outflowing oxygen saturation remained at or above 80% of the inflowing oxygen saturation at that temperature to ensure that oxygen was available to all fish in the respirometer. The outlet saturation levels were maintained by adjusting the water flow rates which were measured 4 times during each trial (Table 4.3). This condition was consistently satisfied in all but one sample period during the 9.7°C experiment where outlet oxygen saturation decreased to 70-75% of the inlet saturation.

Fish weight, flow rate, and inflowing and outflowing oxygen concentrations were used to calculate oxygen consumption as mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$. The experiment was replicated at each temperature with a 3 by 3 Latin Square design such that each density group of flounder was tested in each of the three respirometers (Steel and Torrie 1980). Disturbances such as flow interruptions during daily maintenance procedures occurred during the measurement periods. These

TABLE 4.3

Water flow rates measured during the data collection periods for the calculation of oxygen consumption (N = 12). Flows were increased for higher density groups to maintain an 80% oxygen saturation in the drain water of each respirometer.

Temperature (°C)	Density (kg $\cdot$m^{-3})	Flow rate (L $\cdot$ min^{-1}) ± s.d.
2.2°C	5	2.6 ± 0.6
	25	6.0 ± 1.0
	50	6.9 ± 1.1
9.7°C	5	2.9 ± 0.3
	25	6.9 ± 1.4
	50	7.6 ± 1.7

interruptions were eliminated from the datasets. Analysis of variance (Steel and Torrie 1980) tested the effect of density, temperature, and trial on the oxygen consumption of winter flounder. A tank factor was nested in trial in the analysis. Where factors indicated significant differences, Student Newman-Kuels non-parametric tests were used to test for differences among levels.

4.3 Results.

Oxygen consumption rates (mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$) for each density of flounder measured at the two temperatures are listed in Table 4.4. According to a three-way ANOVA, temperature and density interacted significantly with oxygen consumption ($P<0.05$). As temperature increased from 2.2°C to 9.7°C, mean oxygen consumption rates increased for all density groups. The smallest increase in mean oxygen consumption was in the low density group, and the largest increase was in the medium density group (Table 4.3). Using the SNK non-parametric test, all mean oxygen consumption rates for the 3 levels of density were significantly different at each of the 2 temperature levels.

At 9.7°C, oxygen consumption increased as density increased from 5 kg • m^{3} to 25 kg • m^{-3}, but decreased as density increased from 25 kg • m^{3} to 50 kg • m^{-3}. The highest oxygen consumption rate was for the 25 kg • m^{3} density group (60.9 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$) and the lowest the lowest was for the 50 kg • m^{-3} density group(44.2 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$).

At 2.2°C, oxygen consumption had an inverse relationship to stocking density. The highest oxygen consumption rate was for the 5 kg • m^{3} density group (50.4 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and again the lowest oxygen consumption rate was for the 50 kg • m^{3} density group (28.5 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$).

TABLE 4.4

Mean oxygen consumption rates (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) ± s.d. for winter flounder stocked at 5 kg • m^{-3}, 25 kg • m^{-3} and 50 kg • m^{-3} measured at 2.2°C and 9.7°C.

Temperature (°C)	Density ($kg \cdot m^{-3}$)	Mean oxygen consumption rate ± s.d. ($mg\ O_2 \cdot kg^{-1} \cdot h^{-1}$)
2.2°C	5	50.4 ± 31.9
	25	33.3 ± 11.8
	50	28.5 ± 12.2
9.7°C	5	57.5 ± 37.0
	25	60.9 ± 13.2
	50	44.2 ± 6.7

4.4 Discussion.

The values of the present study are similar to those for Atlantic halibut (Björnsson 1994) and less than those for Japanese flounder (Honda 1988) at high stocking densities. Oxygen consumption rates at 2.2°C for the medium and high density groups (33.3 ± 11.8 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ and 28.5 ± 12.2 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, respectively) were within the same range as the oxygen consumption rates for Atlantic halibut at 7°C (38.3 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$ at 16.5 kg $\cdot m^{-2}$ and 28.5 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$ at 61.4 kg $\cdot m^{-2}$).

The biological observation was that these fish displayed a thigmotaxis. The flounder were most quiescent when in contact with other flounder. The observation made from the data was that as stocking density increased, mean ROC rate decreased. Similar reports on the effect of stocking density on oxygen consumption have developed an index to describe the change in oxygen consumption as stocking density changes. This index is known as "group effect." Imbayashi et al. (1992) provided the formula to calculate group effect. Group effects (GE) (%) were calculated using the equation:

$$GE = \{M_{1MO2} - M_{2MO2}\} \times 100 / M_{1MO2}$$

where M_{1MO2} = oxygen consumption of density group 1 (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$), and M_{2MO2} = oxygen consumption of density group 2 (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$). Using this approach to compare group effects between experimental densities the calculated group effects for winter flounder ranged from 14% to 43%. One exception, the group effect between 5 kg $\cdot m^{3}$ and 25 kg $\cdot m^{-3}$ at 9.7°C was negative (-6%).

The lower oxygen consumption rates in the higher stocking density groups of winter flounder, at both temperatures, represents a group effect similar to that described for Japanese flounder (Honda 1988) and catfish eel, *Plotosus anguillaris*, (Kanda and Itazawa 1981). Grouped flounder which overlap one another had lower oxygen consumption rates because of a thigmotactic response (Honda 1988). Visual contact among fish within a group induced lower oxygen consumption rates for rainbow trout, *Oncorhynchus mykiss*, and medaka, *Oryzias latipes* (Itazawa et al. 1978). The hydrodynamic advantage of schooling and the close proximity of similar species has led to lower oxygen consumption rates in some schooling and non-schooling freshwater species (Parker 1973).

Other species, such as red sea bream, *Pagrus major* (Imbayashi et al. 1992, Imbayashi and Takahashi 1987) and blennies, *Blennius pholis* (Wirtz and Davenport 1976), display opposite group effects. Blennies and young red sea bream are territorial, which suggest that when they are grouped, metabolic rates would be elevated as aggressive behaviour emerged (Imbayashi et al. 1992, Imbayashi and Takahashi 1987, Wirtz and Davenport 1976). Older sea bream, which do not exibit territorial behavior, did not exhibit the same changes in oxygen consumption (Imbayahsi et al. 1992).

The oxygen consumption rates of grouped Japanese flounder (25.4 ± 0.4 mL $O_2 \cdot kg^{-1} \cdot h^{-1}$) were similar to flounder that burrowed in the sand, but were less than the oxygen consumption rates of solitary flounder exposed to a bare surface (30.8 ± 0.5 mL $O_2 \cdot kg^{-1} \cdot h^{-1}$), implying a stress response in fish prevented from burrowing (Honda 1988). The grouped flounder displayed a thigmotactic-like behaviour (Honda 1988). Flatfish in general are thigmotactic; for example juvenile turbot distribute themselves in patches in tanks rather than evenly spread out throughout the tank (Fukuhara et al. 1990). Winter flounder in the present study distributed themselves in similar patches in the low and medium densities. In the large density group, where % coverage of the bottom of the tank was 130%, flounder were always in contact with other flounder which would have induced lower oxygen consumption rates.

Unlike salmonids which develop social heirarchies and display other agonistic behaviours readily (Metcalfe et al. 1995, Christiansen et al. 1991, Kjartannson et al. 1988, Schreck et al. 1985), aggressive behaviour in winter flounder was not obvious. Fish which demonstrate aggression usually have elevated oxygen consumption rates as stocking densities increase (Mazur and Iwama 1993, Christiansen et al. 1991, Brett 1964). Where schooling behaviours occur at higher stocking densities, oxygen consumption rates do not increase significantly with density (Jørgensen et al. 1993). In the present study, little swimming activity occurred in the high density group and less occurred in the other two groups. Schooling behaviour is not a usual component of the behavioural repertoire in flatfish.

The effect of density on production factors such as growth and survival (Björnsson 1994, Martinez-Tapia and Fernández-Pato 1991, Kjartannson et al. 1988, Wallace et al. 1988, Papoutsoglou et al. 1987) indicate optimal stocking densities vary from species to species. High stocking densities inversely affected growth rates in rainbow trout. Trout stocked at 15 kg • m^{-3} had better growth rates than trout stocked above 80 kg • m^{-3} (Papoutsoglou et al. 1987) and similarly trout initially stocked at 107 kg • m^{-3} had better growth rates than trout initially stocked at 219 kg • m^{-3} (Holm et al. 1990). Miller et al. (1995) and Kindschi et al. (1991) provided supplemental oxygen to rainbow trout stocked at 2.5 kg • m^{-3} and 10 kg • m^{-3}, and 30 kg • m^{-3} to 294 kg • m^{-3} respectively, and found feed consumption, feed conversion and oxygen consumption did not differ between high and low density groups. Although growth in the 10 kg • m^{-3} density group was less than the 2.5 kg • m^{-3} density group, total biomass of fish produced was still greater in the 10 kg • m^{-3} density group (Miller et al. 1995). Increased growth rates have resulted for Arctic charr, *Salvelinus alpinus* L., fingerlings stocked greater than 37 kg • m^{-3} (Wallace et al. 1988) and for 2-year-old fish stocked greater than 60 kg • m^{-3} (Jørgensen et al. 1993).

Growth rates in flatfish are influenced by stocking density (Björnsson 1994, Martinez-Tapia and Fernández-Pato 1991, Honda et al. 1988). Japanese flounder reared at 20 kg • m^{-2} (intial weight 90 - 100 g) had growth rates similar to flounder at lower densities (Honda et al. 1988). In turbot, stocking densities of 68 kg • m^{-2} improved food conversion indices and survival but growth was not significantly greater (Martinez-Tapia and Fernández-Pato 1991). The results of the present study indicated that oxygen consumption rates were influenced by stocking density. The interaction between oxygen consumption and growth at different levels of stocking density was not directly examined. Bejda et al. (1992) demonstrated that growth in winter flounder was related to dissolved oxygen concentration. Björnsson (1994) indicated that density groups of Atlantic halibut with greater growth rates had higher oxygen consumption rates and that growth was related to stocking density to a threshold level of 100% coverage of the tank. The highest stocking density in this study covered 130% of the tank (*ie.* more than one layer of the bottom) and had the lowest oxygen consumption rates. Assuming increased growth coincides with an increased oxygen consumption rate, the highest density group in this study would not likely be the most desirable density group for intensive culture of winter flounder. However, the true upper production limit cannot be determined without results of growth rates.

Survival is also variable with stocking density (Miller et al. 1995, Mazur and Iwama 1993, Wallace et al. 1988, Refstie 1977). Mortality rates of Arctic charr and rainbow trout were greater in 5 kg • m^{-3} and 15 kg • m^{-3} density groups (Jørgensen et al. 1993, Wallace 1988, Refstie 1977).

At densities above 60 kg • m^{-3} mortality rates in Arctic charr were reduced (Jørgensen et al. 1993, Wallace et al. 1988) Adult Atlantic salmon, *Salmo salar*, stocked at densities above 125 kg • m^{-3} displayed no chronic stress responses (Kjartannson et al. 1988). In charr, no stress response to high stocking density was due to the inhibition of antagonistic behaviour and stimulation of schooling behaviour (Brown et al. 1992, Wallace et al. 1988). However, higher stocking densities increase the risk factor in intensive systems. In the event of water shortage or reduced supplemental oxygen, extreme mortality spikes result (Miller et al. 1995, Martinez-Tapia and Fernández-Pato 1991).

The highest oxygen consumption rates in this study were found in the 25 kg • m^{-3} density group measured at 9.7°C (60.9 mg O_2 • kg^{-1} • h^{-1}). This was interesting because this density group has the smallest average fish size (Table 4.1). As fish grow larger, their oxygen consumption per unit of body weight decreases (Waller 1992, Kikuchi et al. 1990, Jobling 1982). It is not surprising that this density group had the higher oxygen consumption rate and our value may reflect the differences in size in this study.

Voyer and Morrison (1971) determined that winter flounder consume oxygen independently of dissolved oxygen concentration until oxygen tension is 30% of full saturation. In this study, flow rates were altered to maintain 80% dissolved oxygen saturation in the outflowing water of each respirometer. Reduced oxygen consumption rates at higher densities suggest that supplemental oxygen, as offered to rainbow trout by Miller et al. (1995) and Kindschi et al. (1991), may not be required for intensive culture of winter flounder, other than as an emergency supply.

5. GENERAL DISCUSSION

Successful intensive, marine culturing operations must meet requirements imposed by general physical and biological principles of fish physiology. Physical requirements dictate the quality of the facility with respect to environmental and mechanical factors. Specifically, environmental factors include dissolved oxygen, temperature, salinity, carbon dioxide, pH, and ammonia. These must remain within safe levels. Mechanical factors include water flow rates and tank designs. Problems in both environmental and mechanical factors will affect the production of fish at the facility.

Biological factors determine the quality of the product with respect to somatic and production factors. Somatic factors include growth rate and feed conversion ratios, and should be carefully monitored throughout the grow-out cycle. Production factors such as stocking density and feeding rates regulate the productivity of the farm.

The essential aerobic requirements of various pleuronectids which are being considered as new species for culture in Atlantic Canada need elucidation. Three factors which affect the culture of flatfish were studied for their affects on oxygen consumption. The primary objective was to determine the routine metabolism of cold water pleuronectids under land-based aquaculture holding conditions, comparing performance of species, and the influences of body size and stocking density. The experiments were repeated during 3 seasons and conducted at seasonal water temperatures.

Traditional flow-through respirometry techniques were modified for use with groups of fish in tanks simulating aquaculture holding conditions allowing determination of oxygen consumption.

This study utilized measurements of routine oxygen consumption rates as compared to standard and active rates, which proved to be the most practical oxygen consumption for aquaculture. Estimates of oxygen consumption under culture conditions are often higher than estimates conducted under laboratory conditions (Brown et al. 1984). Respirometry chambers which simulate aquaculture holding tanks allow flounder the freedom to move within the respirometry chamber without forced swimming. Recording mean oxygen consumption rate over a day and night period allowed calculation of a value representative of oxygen demands during a 24 h period. This was the primary advantage of our technique because the data more accurately represented conditions in the rearing environment compared with alternative methods such as recording only minimum or maximum oxygen consumption values. Changes in oxygen consumption due to daily activities were expressed in the standard deviation of the mean.

Several questions concerning the metabolism of fish in land-based aquaculture can be addressed using the single pass, flow-through respirometry technique developed in this study. Comparisons among species can be identified and quantified under identical test conditions. Variables such as temperature, fish size and stocking density can be independently altered to determine effects on respiration.

Changes in fish size affect performance during the grow out phase of aquaculture production. Fish size is generally related to oxygen consumption exponentially manner (Jobling 1994, Jobling 1982, Zeuthen 1953) with larger fish using less oxygen per unit of body mass. However, over the restricted size range utilized in this study, the relationship between fish size and oxygen consumption was better explained with a linear model. Evaluating the effect of fish size over a broader range of sizes (*eg.* 1 - 1000 g) would likely illustrate the exponential relationship of fish size and oxygen consumption. This will be an important component when modelling water flow regimes appropriate for each stage of the culture process (*eg*. hatchery phase and on-growing phase).

A broad range of temperatures was encountered in this study because these experiments were replicated during several seasons in ambient seawater of the Bay of Fundy. The optimal temperature for flounder culture is still undetermined but the range of temperatures has been narrowed. In one experiment the oxygen consumption rates of yellowtail and winter flounder increased as temperature increased to 11°C. Assuming the response in oxygen consumption reflected a similar response in growth, increasing water temperature from 2°C to 11°C would improve performance. During another experiment the highest observed oxygen consumption rates were at 9°C for winter flounder. The temperature where oxygen consumption, and presumably growth, is optimal was not specified from these studies, but these studies suggest it is 9 - 11°C.

Apparently a parabolic response in oxygen consumption occurred between 2 - 14°C. This trend was similar to that found for turbot at 8 - 24°C (Waller 1992). The optimal temperature for turbot is 16 - 19°C, reflected in maximum rates of oxygen consumption and growth (Imsland et al. 1995, Waller 1992).

Optimal stocking density is always difficult to determine. At this stage in the study of culture requirements of pleuronectids, the measurement of oxygen consumption without feeding precluded the simultaneous measurement of oxygen consumption and growth. Oxygen consumption was inversely related to stocking density as stocking density increased from 25 kg • m^{-3} to 50 kg • m^{-3}. Maintaining the earlier assumption that increased growth rates were associated with increased oxygen consumption rates, the best growth responses should have occurred in the lowest density group. Tanks with low stocking densities have high production costs in intensive land-based aquaculture. The intermediate stocking density group may be a more reasonable density to grow pleuronectids where costs of additional construction and pumping water is recovered with better growth rates.

Information on the growth rates of flounders at all stages of ontogeny is essential to predict with confidence which conditions are optimal for culture of these species. Studies of growth rates of flounders in groups are the logical extension of the results of these studies, as performed by Björnsson (1994), Fonds et al. (1992) and Honda et al. (1988). The optimal conditions for pleuronectid performance in intensive, land-based culture systems will be defined based upon growth rate information and the results presented herein. Similar studies have indicated the optimal stocking density and rearing temperature for various sized Atlantic halibut, *Hippoglossus hippoglossus*, (Björnsson and Tryggvadóttir 1996, Björnsson 1994) and the optimal stocking density for Japanese flounder (Honda et al. 1988).

This comparative approach is critical in selecting the most profitable species for exploitation. Both the species studied and physical variables, the most critical being temperature, were studied herein.

The yellowtail flounder is a promising aquaculture candidate in cold environments which warm up moderately during the summer. Increased aerobic metabolism from 2 - 11 °C and active feeding throughout the year under culture conditions suggested that the yellowtail flounder has suitable characteristics for these environments. Similar responses in oxygen consumption were observed for winter flounder over the same temperatures, but active feeding was suspended at winter temperatures below 5 °C. The winter flounder may be better suited to environments where winter temperatures are higher. For both species, the optimal rearing temperature is below 14 °C.

Significant differences in mean oxygen consumption rate (mg $O_2 \bullet kg^{-1} \bullet h^{-1}$) were found among the three species tested at each temperature. The differences between winter flounder and yellowtail flounder may not be biologically different when the mean oxygen consumption rate of these two pleuronectids is contrasted to salmonids already in intensive land-based culture conditions (Jørgensen et al. 1993, Fivelstad and Smith 1991). Mean oxygen consumption rates were within the same range as other flatfish under culture conditions (Davenport et al. 1990, Brown et al. 1984). Therefore, the conclusions made on the influence of fish body size and stocking density on the oxygen consumption of winter flounder were considered equally true for yellowtail flounder.

Two major assumptions were made throughout these experiments. First, all experimental fish were considered healthy. All flounder used in these studies were wild-caught fish, and health inspection upon capture was based on external examination. Throughout the experimental periods, flounder holding tanks were checked daily and any mortalities were promptly removed. Flounder selected for oxygen consumption studies were actively feeding fish and were in good condition externally.

The second assumption regards the digestive process of the flounder in the respirometry studies. While in holding tanks, flounder received daily feed rations. Immediately prior to the measurement of oxygen consumption, time was allowed for complete digestion and assumed evacuation of the gastrointestinal tract. Between replications, flounder were offered one feed ration, but whether this ration enabled the flounder to return to pre-feeding metabolism was not clear.

American plaice and yellowtail flounder were actively feeding at the colder temperatures in holding tanks. However, oxygen consumption rates for these species at 2°C were similar to the oxygen consumption rate of winter flounder which did not actively feed at the cold temperatures. Therefore, digestion by flounder in respirometry tanks was complete and the oxygen consumption rates measured in the respirometers were non-feeding rates.

Increased oxygen consumption rates in poikilotherms are associated with increased temperatures (Jobling 1982, Voyer and Morisson 1971), feeding (Fonds et al. 1992, Saunders 1963), greater growth rates (Björnsson 1994, Bejda et al. 1992) and elevated levels of plasma cortisol and haematocrit (Mazur and Iwama 1993). In the present study, the effects of temperature was monitored and feeding was controlled for, but it is difficult to state which of the remaining factors led to the increases observed in oxygen consumption. However, flounder usually are on the bottom surface and not actively swimming (Gibson 1975) except when feeding (Tyler 1971). Stress levels in experimental flounder were considered low because flounder were eating soon after moving flounder back into holding tanks. Each experiment lasted approximately 10 days; the influence of growth would be minimal. Overall, the effect of the remaining factors was not a concern in this study.

Fish were handled during transfer from holding to experimental tanks. Increased metabolism is often associated with handling stress. However, flounder quickly ceased swimming activity after handling, perhaps selecting a camouflage strategy. For example, yellowtail flounder moved from the respirometer chamber and into 3 m diameter holding tanks, remained quiescent and after 30 min were feeding on dry feed, a good indication of minor to no stress response.

To understand and select the requirements for a commercial, land-based production facility for flounders, there must be knowledge of all components which affect the success of the facility.

The supply of water, utilization of feed, removal of wastes, and other requirements are essential to the design of an operation. The most important recommendation from this thesis is that the minimal routine oxygen requirements for non-feeding pleuronectids during the on-growing phase of intensive production range from 28.5 to 292 mg $O_2 \bullet kg^{-1} \bullet h^{-1}$. Daily waterflow requirements should be sufficient to meet the oxygen demands identified in this study.

Both the yellowtail flounder and the winter flounder are species which appear adaptable to intensive rearing in land-based tanks. The lower oxygen consumption rates measured in flounder compared to Atlantic salmon measured under similar conditions (Fivelstad and Smith 1991) clearly suggested that lower aeration costs may be a potential advantage of flounder culture.

The optimal temperature for rearing flounder was within the temperatures tested during these studies. Temperatures above 13°C (and 14°C) were above the optimal metabolism for the three species studied. For winter flounder, temperatures at or below 2°C were below the optimal metabolic temperature. Yellowtail flounder and American plaice displayed different responses in experiments repeated at various temperatures.

As fish grow a lower oxygen demand per unit of body weight is an interesting biological aspect. Fish approaching market size (*eg.* 600 - 800 grams) require 56% less oxygen (per unit of body weight) and therefore less waterflow required by an equal biomass of juvenile fish (*eg.* 100 g).

Stocking densities similar to those presently used in culture of other flatfish like Atlantic halibut and turbot are likely appropriate for the culture of pleuronectids. However, in the absence of data on growth rates, an accurate prediction of optimal stocking density cannot be made.

These initial studies identified several new questions involving the relationship of oxygen consumption and the aquaculture production performance of pleuronectid fish. The primary question is the relationship of the growth rate of flounder to oxygen consumption. A secondary topic would focus on specific measurements of oxygen consumption over more intermediate temperatures to isolate the optimal temperature for metabolism of each of these pleuronectid species. Finally, measurements of oxygen consumption over a broader range of fish size, from weaned juveniles to large market size animals, will refine the biological relationship between fish size and oxygen consumption in post-metamorphosed flounder.

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