THE METABOLIC AND CARDIAC RESPONSES OF CUNNER (Tautogolabrus adspersus, WALBAUM) TO TEMPERATURE FLUCTUATIONS

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by

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

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Memorial University of Newfoundland

March, 2007

St. John's Newfoundland

Your file Votre référence
ISBN: 978-0-494-31243-8
Our file Notre référence
ISBN: 978-0-494-31243-8

Abstract

The cunner, a marine teleost, exhibits winter dormancy (i.e. becomes inactive and stops feeding) when seawater temperatures fall below 5°C. To examine if this dormant state is associated with active metabolic depression, the effect of season on routine metabolic rate (MRr) and cardiac function was examined in this species. Fish were kept under ambient conditions for a year and their MRr determined at 5 critical seasonal temperatures (5, 9, 14, 5 and 0°C), while cardiac function was determined at the 3 last temperatures (14, 5 and 0°C). Moreover, the ability of cunner to adjust MRr, and cardiac function, when challenged with acute temperature changes was also assessed. This was accomplished by exposing cunner, acclimated to either 5 or 0°C, to a decrease/increase of 5°C, respectively, and maintaining the fish at the final temperature for 3 additional hours. Cunner seasonally depressed MRr and cardiac function, showing high Q_{10}s (> 4) between 5 and 0°C. Further, when subjected to an acute temperature increase (0 to 5°C), dormant cunner rapidly increased both MRr and cardiac function, with high Q_{10}s (> 5). However, when subjected to an acute temperature decrease (5 to 0°C), the cunner were able to actively depress MRr (Q_{10}~8), but not cardiac function, showing a decoupling of the MRr and cardiac response.

Acknowledgements

First and foremost I would like to express gratitude to my supervisors, Dr. Kurt Gamperl and Dr. Bill Driedzic, not only for taking me on as a student, but for all the guidance and support during the last two and half years.

I also want to thank the OSC and MUN Technical Services for their fast response whenever a problem with the equipment appeared, and to thank Daryl Jones for his help with animal husbandry. I would also like to extend my appreciation to Animal Care services, especially Ms. Pat Barker, for providing me with the blood necessary for the calibration of the flow probes, as well as, to Dr. Paul Snelgrove and Dr. Don Deibel for the use of cold room facilities, and to Dr. David Schneider for statistical advice.

I would also like to thank my lab mates, from both the Gamperl and Driedzic labs, as well as to the entire BBQ crowd, in particular Paula Mendonça, Johanne Lewis and Jennifer Monk for all their help, support or just for good times these past years.

At last I want to thank Darek Moreau, my family and friends in Portugal for their continuous support. Finally, a huge thank you to my mom Vitória that always supported me in every way she could, and to whom all my achievements are dedicated.

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List of Abbreviations

ATP - Adenosine tri-phosphate

CO_2 – Carbon dioxide

$Co_{2(i)}$ - Oxygen concentration of inflowing water

$Co_{2(o)}$ – Oxygen concentration of outflowing water

f_H - Heart rate

M – Mass

MO_2 - Oxygen consumption

MRr - Routine metabolic rate

MS-222 - Ethyl-*m*-aminobenzoate

NaCl – Sodium chloride

Q - Cardiac output

SV_H - Stroke volume

Vw – Water flow rate through the respirometer

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1. General Introduction

Thermal fluctuations and the effect on animals

The effect of temperature on biological processes can be determined using the temperature coefficient Q_{10}, which describes the effect of a 10°C change in temperature on the rate of a particular process (Rome et al., 1992). Chemical and biological processes are extremely temperature sensitive having Q_{10}s that generally range from 1.8 to 4 (Rome et al., 1992). Metabolic rate, commonly measured as the rate of oxygen consumption, is also affected by temperature with Q_{10} values for most species of poikilotherms, and temperature ranges, between 2 and 3 (Rome et al., 1992; Guppy and Withers, 1999).

As mentioned above, temperature fluctuations affect virtually all levels of biological organization, including: 1) rates of molecular diffusion and biochemical reactions; 2) membrane permeability; 3) cellular, tissue and organ function; and 4) their integration in the whole organism. Further, temperature shifts may alter the equilibrium of several biological processes such as the synthesis and degradation of biological structures, organism metabolic requirements as well as trophic interactions (Guderley and St-Pierre, 2002). Thus, many species regulate their body temperature to avoid the deleterious effects of temperature fluctuations on physiological and metabolic processes, the degree of regulation varying with the animal's thermoregulatory mode.

Types of thermoregulation

The thermoregulatory mode of animals can be broadly classified into two categories. *Endothermic* animals achieve considerable independence from their habitat by conserving much of their metabolically generated heat. Mammals and birds are examples of this group, which produce

metabolic heat at high rates, have a relatively low thermal conductivity, and regulate their body temperatures within relatively narrow limits. *Ectothermic* animals on the other hand, have their body temperature determined primarily by the heat gained from, and lost to, their environment. They also produce heat metabolically, but at a considerable lower rate, and usually have high thermal conductivities (Guderley and St-Pierre, 2002; Guderley, 2004).

The majority of ectotherms can also be considered *poikilothermic* organisms, meaning that their body temperature undergoes considerable variation, usually in response to changes in environmental temperature. In contrast, *homeothermic* organisms are able to maintain their body temperature very stable. However, not all poikilotherms undergo variations in body temperature. The body temperature of Antarctic fish, for example, is relatively constant due to the almost non-existent thermal variation of their habitat (Guderley, 2004).

Another important aspect of ectothermic thermal biology is that while the body temperature of terrestrial ectotherms can differ from their immediate habitat, in the aquatic environment ectotherms (e.g. frogs, turtles and the majority of fish) body temperatures are very similar to that of the water (Guderley and St-Pierre, 2002). The fact that most fish breathe water, and that water has a high heat capacity, makes heat exchange very fast. Moreover, the arrangement of the gills and blood vessels in fish leads to rapid thermal equilibration with the environment, making their body temperature very close to ambient temperature (Guderley, 2004; Crockett and Londraville, 2006).

Although ambient temperature in aquatic environments does not vary to the same degree as in terrestrial habitats, the ocean surface temperature still ranges from -2°C (in the polar seas) to over 30°C (in the tropics). Further, in temperate-zones, daily (5 to 35°C in tidewater pools) and seasonal temperature fluctuations (e.g. -0.5 to 14°C in Logy Bay, Newfoundland, Canada) can be substantial

(Bridges, 1988; Levesque et al., 2005). Therefore, the study of fish thermal biology is quite complex, since, as mentioned above, they can be both ectotherms and homeotherms with even some species (e.g. tuna and swordfish species) capable of some degree of regional heterothermy (maintaining certain portions of the body at a higher temperature than the rest) (Guderley, 2004).

Strategies to cope with thermal fluctuations

Animals that are not able to cope with environmental thermal fluctuations either remove themselves from the unfavourable environment (e.g. migration), or employ a combination of behavioural and physiological adaptations that allow them to cope with the particular environmental stress (Storey, 2001).

Non migratory fish, for example, usually undergo one of the following physiological adjustments when faced with seasonal cooling (Guderley and St-Pierre, 2002; Guderley, 2004):

1) A slow down of their physiological processes by submitting to the Q_{10} effects;

2) An offsetting of Q_{10} effects by maintaining function/capacity using compensatory mechanisms;

3) An enhancement of Q_{10} effects on physiological processes, by reducing standard metabolic rate to a greater extent that would be predicted by a Q_{10} effect alone. A phenomenon called metabolic depression.

Metabolic depression

Metabolic depression is a reduction in metabolic rate below normal resting values. It has been recorded in all major animal phyla as a response to numerous environmental stresses including

low temperature, desiccation (lack of water), hypoxia/anoxia (lack of oxygen), hypersalinity and food deprivation. The extent of metabolic depression varying from 60% of the resting metabolic rate to complete absence of measurable metabolism (for reviews, see Storey and Storey, 1990; Guppy et al., 1994; Hand and Hardewig, 1996; Guppy and Withers, 1999; Storey and Storey, 2004).

The first known observations regarding hypometabolism were noted during the pioneering microscopical work of van Leeuwenhoeck 1702 (*in* Guppy 2004) that identified small desiccated (dormant) animals (rotifers) in dry sediments that resumed normal activities when rehydrated, a phenomenon called cryptobiosis. A substantial body of work has been completed since the dormancy of rotifers was described, and it is now accepted that the mechanisms responsible for metabolic depression are found at the cellular level (Jackson, 2000; Boutilier, 2001). Although the mechanisms that trigger metabolic depression are still poorly understood, a considerable amount of work has been done to understand the mechanisms that are involved in regulating and coordinating metabolic depression in hypometabolic systems. Basically, a coordinated decrease in the ATP (adenosine tri-phosphate) supply and demand is necessary for metabolic depression to be achieved. Cells are able to do this by decreasing or switching to more effective energy production (e.g. carbohydrate degradation) and energy consuming processes (e.g. ion pumping and protein synthesis\degradation) (Guppy et al., 1994; Boutilier, 2001; Storey and Storey, 2004).

Animals that undergo metabolic depression are generally said to be in a state of torpor or hibernation. Hibernation/torpor is a winter survival strategy, widely used by animals in order to reduce the costs of maintaining a large thermal gradient between the body and the ambient environment (e.g. mammals) and/or deal with decreasing food availability (Hand and Hardewig,

1996; Storey and Storey, 2004). The term torpor is usually reserved for the group of true mammalian hibernators and birds, and has been defined as a controlled hypometabolic state with body temperatures regulated at a low level and with a typical energetically expensive arousal that leads to a rapid increase in body temperature to its normal operative range (Ultsch, 1989). There are several patterns of torpor that can be exhibited by animals depending on the duration and extent of the metabolic/temperature depression. Hibernation or prolonged torpor may last weeks to several months, and in this state the animals do not feed. In contrast, daily torpor usually only lasts a few hours and is interrupted by daily foraging and feeding bouts (Geiser, 2004).

Ectothermic animals

In ectotherms, the term torpor or hibernation is not so readily used to describe the physical state induced by metabolic depression. Some authors feel that ectothermic animals do not show a true state of 'torpor', as it is defined for mammalian hibernators, because there is no controlled decrease in body temperature. The term dormancy was defined by Crawshaw (1984) as a behaviourally inactive state, shown by adult reptiles, amphibians and fish, as a response to low temperature, lack of water and/or low food availability. Dormant animals usually do not feed and greatly decrease their level of spontaneous activity, usually hiding or burying themselves (Crashaw, 1984). Thus, the term dormancy does not connote other conditions such as unusually low body temperature; however, it can be used to describe the behavioural state of ectotherms that show active metabolic depression ($Q_{10} > 3$).

Several species of reptiles and amphibians are known to deeply depress their metabolism during winter. In order to avoid freezing temperatures during the winter these animals often take up

residence in lakes and ponds that eventually become anoxic due to ice coverage (Donohoe et al., 1998; Jackson, 2000). The turtle *Chrysemis picta bellii* is among the best studied ectotherms that actively depress their metabolism during winter. It has been reported to survive anoxia for several months at low temperatures (< 5°C) by entering a comatose-like state where basal metabolism is depressed by 90% (Jackson, 2000; Boutilier, 2001). Similarly, the frog *Rana temporaria* tolerates hypoxia/anoxia at very low temperatures by actively depressing its metabolism by 40% (Donohoe and Boutilier, 1998; Boutilier, 2001).

Metabolic depression in fish

The overwintering response of fish, as in most vertebrates, can be broadly grouped into two ecological categories: 1) fish that remain active throughout the winter months and continue feeding (e.g Salmonidae); and 2) fish that become inactive and spend their winter months in a state of dormancy (Crawshaw, 1984; Ultsch, 1989) that may be associated with metabolic depression (e.g. some species of Anguillidae, Centrarchidae, Ictaluridae and Labridae).

The majority of research regarding metabolic depression in fish has been done on aestivating fish, the dipnoans or lungfish, and hypoxia/anoxia resistant species. In order to avoid desiccation, the lungfish excavates a burrow in the drying mud where it stays for at least 9 months. Over 60-120 days, oxygen consumption (MO_2) falls by 85% and breathing, heart rate and blood pressure decrease significantly (Guppy et al., 1994). The crucian carp (*Carassius carassius*), and its close relative the goldfish (*Carassius auratus*), are both known to survive hypoxia/anoxia in ice-covered lakes and ponds for several months at low temperatures (overwinter; < 5°C). However, in contrast to turtles and frogs, the crucian carp maintains some level of activity during this period due to other

hypoxia/anoxia survival strategies (e.g. haemoglobin with extreme affinity for oxygen, changes in gill morphology and production of ethanol to avoid lactate accumulation); causing the degree of metabolic depression in these fish to be less than what is shown by turtles (Van Waversveld et al, 1989; Johansson et al., 1995; Nilsson et al., 2004).

Studies regarding metabolic depression caused solely by low temperature exposure are relatively scarce. Crawshaw (1984) studied dormancy in two teleost species, the largemouth bass (*Micropterus salmoides*) and the brown bullhead (*Ictalurus nebulosus*). Both species hide and show low levels of spontaneous activity at low temperatures (approximately 3°C), as well as decreased metabolic rates, respiratory and cardiac frequencies. However, the authors report that it is not clear whether this dormant state is due to an active depression of metabolic rate. On the other hand, Walsh et al. (1983) studied the American eel (*Anguilla rostrata*), another teleost that exhibits winter dormancy (inactivity) when water temperatures drop below 8°C. The authors found that between 10 and 5°C the eel's metabolic rate shows a Q_{10} = 4.1, an indication of active metabolic depression as a response to low temperatures. More recently, Sayer and Davenport (1996) reported that the goldsinny wrasse (*Ctenolabrus rupestris* (L.)), a marine teleost, spends the winter months in a dormant state. Further, when acutely challenged with a temperature decrease of 8 to 4°C, the goldsinny showed a large decrease in metabolic (Q_{10} = 37.2), heart (Q_{10} = 10.8) and ventilation rate (Q_{10} = 6.7).

2. Study Rationale

Studies of metabolic depression in fish are very limited. Furthermore: 1) the majority of research has focused on metabolic depression as a response to lack of water (desiccation) and oxygen (hypoxia/anoxia), or to a combination of low oxygen and temperature (Smith, 1930; Van Waversveld et al, 1989; Johansson et al., 1995; Muusze et al., 1998; Nilsson et al., 2004); and 2) the few studies that have examined low temperature induced metabolic depression (Walsh et al., 1983; Crawshaw, 1984; Sayer and Davenport, 1996) did not focus on seasonal (i.e. temperature and photoperiod) conditions.

The cunner (*Tautogolabrus adspersus*; Figure 2.1), a member of the Labridae, is distributed in the Western Atlantic from Newfoundland and the Gulf of St. Lawrence in Canada to Chesapeake Bay in the USA, (Scott and Scott, 1988), and exhibits winter dormancy in response to the low environmental temperatures of its habitat during winter (Green and Farwell, 1971). For example, along the coast of Newfoundland cunner remain in shallow water throughout the year, rather than retreating to deeper warmer waters as observed in other Newfoundland coastal species (Green and Farwell, 1971). Further, they occupy small home ranges (Green, 1975), become dormant in late fall/early winter when the ocean temperature is around 5°C, and 'arouse' from this state during the spring when the ocean temperature returns to 5°C (Green and Farwell, 1971). The temperature of the ocean surface along the coast of Newfoundland remains below 5°C for nearly 6 months of the year, 3 of which are around 0°C, making it a relatively long and cold winter for the local non-migrating species (i.e. the cunner).

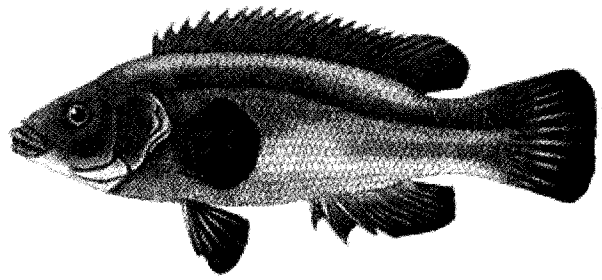


Figure 2.1 – The cunner, *Tautogolabrus adspersus* (Walbaum). Source: http://www.fishbase.org

The only two metabolic studies on cunner were performed in a more southerly population (Woods Hole, New England, USA). The first study goes back 60 years, when Haugaard and Irving (1943) showed that the metabolic rate of winter acclimated cunner (at 5ºC) was about one fourth of the metabolic rate of summer acclimated fish (at 20-21ºC), a Q_{10} of about 2.5. In contrast, Curran (1992, unpublished Ph. D. thesis) reports that cunner from the same population actively decrease routine metabolic rate (Q_{10} = 8.5) when the seawater temperature drops from 12 to 6ºC; remaining dormant for 4 months and maintaining a constant metabolic rate when temperature falls from 6 to 3ºC.

Cunner, in Newfoundland, are at the limit of their Northern distribution and, as previously mentioned, exposed to lower temperatures for longer periods when compared to the southern populations. Further, recent work by Lewis and Driedzic (2007) shows that cunner in Newfoundland greatly depress protein synthesis, in several tissues, while in this dormant state (Q_{10} = 6.7 to 21). Given the above data, it is clear that these fish might be an extremely valuable model for studying several aspects of low temperature-induced metabolic depression in fish.

Therefore, the major goals of this study were: 1) to determine if, and to what extent, cunner in Newfoundland seasonally depress routine metabolic rate and cardiac function; and 2) to determine the ability of cunner to adjust routine metabolism and cardiac function when challenged with acute temperature changes.

A study of cardiac function, in addition to whole-animal metabolism, was performed for several reasons. First, temperature is one of the most important environmental factors influencing cardiac function (Driedzic and Gesser, 1994). Second, cardiac function is a primary determinant of oxygen consumption (i.e. metabolic rate). Finally, very little is known (except for a few Arctic and Antarctic species, see Axelsson, 2005) about the cardiovascular function of teleost fishes at very low temperatures (< 2°C).

3. Materials and Methods

3.1 Experimental animals

Wild cunner (*Tautogolabrus adspersus*) were captured via hoop net in Portugal Cove, Newfoundland, and held at the Ocean Sciences Centre (Memorial University of Newfoundland) for several months prior to the experiments. The fish were maintained indoors in fiberglass tanks (1m square by 0.5m deep) supplied with aerated seawater at seasonally ambient temperature and exposed to a natural photoperiod. Non-dormant fish were fed twice a week to apparent satiation with chopped herring (*Clupea harengus*). Dormant fish did not normally feed, but some chopped herring was offered twice a month, during this period, and removed after 1 day if not consumed. Feeding was suspended from 2 days (active fish) to 2 weeks (dormant fish) before experimentation. The average mass and length of the several groups used throughout the experiments are shown in Appendix A and respective figures.

All experiments were performed in a temperature controlled room and a natural photoperiod was maintained.

3.2 Measurements of Routine Metabolic Rate

3.2.1 Respirometer and Oxygen Consumption Measurements

The minimal metabolic requirements of a fish can be defined using two terms. Standard metabolism is the minimal metabolic rate required to sustain life (i.e. when the animal is spending no energy for activity, digestion, reproductive development, growth or stress responses), whereas, routine metabolism is the metabolic rate of a quiescent fish including some spontaneous movements (Cech, 1990). Oxygen consumption (MO_2) of the cunner was measured using a

custom-built flow-through respirometer. This respirometer allowed for some spontaneous movement, and therefore, MO_2 measurements were considered to be representative of routine metabolic rate (MRr) (Cech, 1990).

The respirometer was a 15 x 20 x 40cm Plexiglass® box with a securely bolted lid (11.875L in volume) that contained a small submersible pump (model MP-10, ZooMed Aquatic), to ensure adequate mixing, and was surrounded by a water jacket for temperature control (Fig. 3.1).

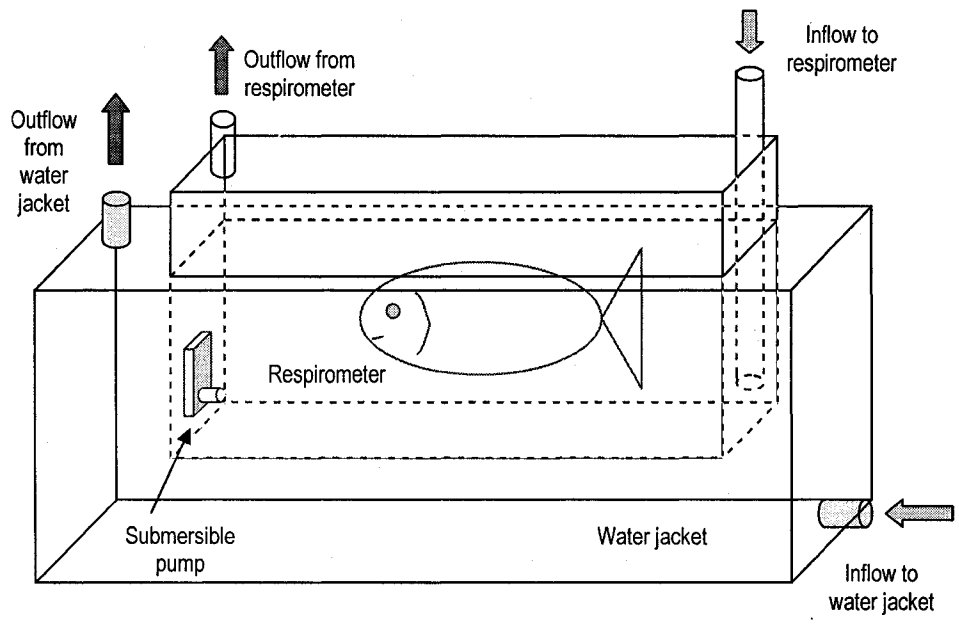


Figure 3.1 – Plexiglass ® chamber for measuring cunner oxygen consumption. See text for details.

The water in the respirometer was continuously circulated using a submersible pump (model NK-1, Little Giant Co., USA) located in a 135L reservoir filled with temperature controlled, air saturated seawater. A second submersible pump (model NK-1, Little Giant Co., USA), also located in the reservoir, was used to continuously circulate water in the water jacket. Water flow rate

through the respirometer (Vw) varied between seasons/measurement periods but was constant within a particular measurement period. Water temperature in the reservoir was maintained by a refrigerating/heating circulating bath (model 1150S, VWR International) and titanium heat exchange coils.

Oxygen concentration was monitored through a circuit composed of tubing with extremely low gas permeability (Gas permeability = 60 and 124 ml O_2 x mm / ([cm^2 x sec x cm Hg] x 10^{-10}) for Tygon® Food and LFL, respectively, Cole Palmer, Inc., USA) that allowed continuous monitoring of water temperature and oxygen concentration of either the inflowing or outflowing water. Oxygen concentration was measured using a galvanic oxygen electrode with thermal sensor (model CellOx 325, WTW, Weilheim, Germany) housed in a flow chamber (D201, WTW, Weilheim, Germany). This oxygen electrode was connected to an oxygen meter (model Oxi340i, WTW) with automatic temperature compensation that was connected to a laptop computer equipped with WTW Multilab® Pilot 4.34 (Fig. 3.2). Normally, the oxygen concentration of the outflowing water ($Co_{2(o)}$) was continuously monitored. However, to make periodic measurements of the oxygen concentration of the inflowing water ($Co_{2(i)}$), the outflowing water was diverted past the oxygen electrode, and back to the reservoir (see valves A and B, figure 3.2), thus providing the oxygen electrode with reservoir water via a peristaltic pump (Masterflex L/S model 77200-12, Cole-Palmer). This way, frequent measurements of $Co_{2(i)}$ were possible without disturbing the water flow rate through the respirometer. Whenever a fish was inside the respirometer a black plastic cover was used to minimize visual disturbance.

Routine metabolic rate (MRr) was calculated as:

$$MRr = \frac{[\,(Co_{2(i)} - Co_{2(o)}) \times Vw\,] \times 60}{M}$$

Where,

MRr - Routine metabolic rate (mg O_2 kg^{-1} h^{-1});

$Co_{2(i)}$ - Oxygen concentration of inflowing water (mg O_2 L^{-1});

$Co_{2(o)}$ - Oxygen concentration of outflowing water (mg O_2 L^{-1});

Vw - Water flow rate through the respirometer (L min^{-1});

M - Mass of fish (kg) (Cech, 1990; modified)

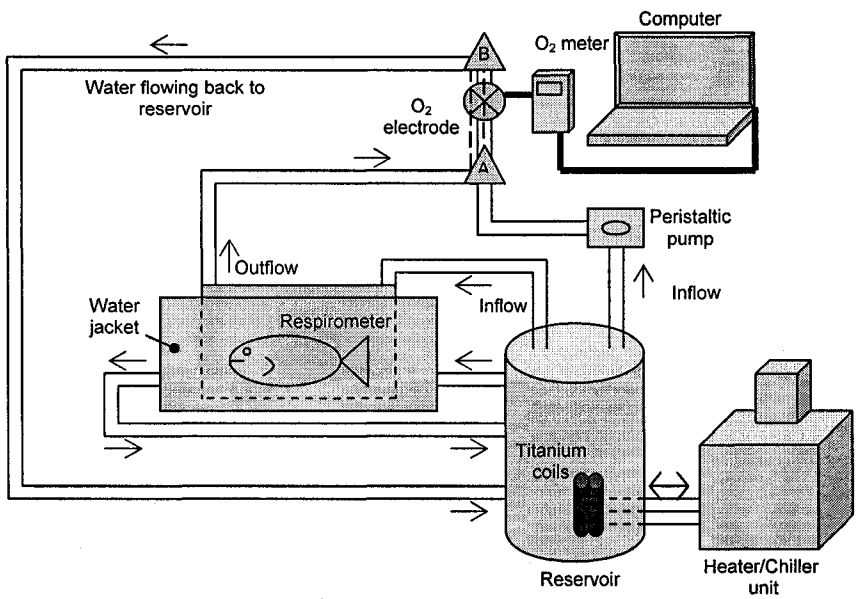


Figure 3.2 - Diagram of the experimental set-up used to measure routine metabolic rate. See text for details.

Whenever MRr was calculated $Co_{2(i)}$ and $Co_{2(o)}$ were continuously measured for 5 minutes (one measurement every 30 seconds) and averaged, to obtain a value of $Co_{2(i)}$ and $Co_{2(o)}$ for that measurement point. Water flow rate through the respirometer (Vw) was measured several times during each trial by weighing the volume of water flowing through the respirometer (outflow), during one minute. Water flow rate and $Co_{2(i)}$ were not measured during the night. However, the last measurement performed in a given day and the first measurement of the next day, as well as measurements performed throughout the day, never showed any variation in either Vw or $Co_{2(i)}$. Therefore, night MRr values were calculated using a continuous recording of $Co_{2(o)}$ and averaged $Co_{2(i)}$ and Vw values; using the last measurement of a day and first of the next day.

Before placing the fish in the respirometer, and immediately after its removal, background oxygen consumption measurements were made. These measurements were intended to be used to correct for any oxygen consumption related to bacterial growth, in the respirometer, during the trial. However, background oxygen consumption was never more than 1-2% of the fish's routine metabolic rate, and therefore considered negligible. Due to differences in fish mass between several of the experimental groups (see Table A.1), and that metabolism scales allometrically, not isometrically, with body mass (Clarke and Johnston, 1999), MO_2 values were converted to mass-independent values using a mass exponent of 0.83. This value is an average of the mass exponents of 3 North Atlantic teleost species (range 0.818 to 0.834) (Killen et al., in press).

Q_{10} values for MRr were calculated as follow:

$$Q_{10} = (R_2 / R_1)^{[10/(T_2 - T_1)]}$$

Where: R_1 = Rate at T_1 R_2 = Rate at T_2

T_1 = Initial temperature T_2 = Final temperature (Rome et al., 1992)

3.2.2 Seasonal Effects on Routine Metabolic Rate

This experiment involved making MRr measurements, on the same individual fish, at 5 selected periods over the course of the annual water temperature cycle (Table 3.1 and Fig. 3.3).

Table 3.1 – Measurement periods for the seasonal routine metabolic rate experiment. Measurement periods were selected based on seasonal ocean water temperatures and the field observations of Green and Farwell, 1971).

Measurement periods	**Time of year**	**Temperature ± 0.5 (°C)**
I	Spring	5
II	Spring/Summer	9
III	Summer	14
IV	Fall	5
V	Winter	0

Periods I and IV corresponded, respectively, to 'arousal' from dormancy (early spring) and the time of year (late fall) when the fish enter their dormant state; both periods when water temperature was approximately 5°C. Periods II (9°C) and III (14°C), late spring and summer, represented periods when the fish were active. Finally, period V was winter, when water temperatures are ~0°C and the fish are in a dormant state. In general, a measurement period would only start after the fish were at the desired temperature for 1.5 to 4 weeks. This short 'acclimation period' (1.5 weeks) at some of the temperatures was unavoidable, due to the short time interval between these

temperatures during the seasonal cycle, and that it took approximately 3-4 weeks to complete a set of measurements at each temperature.

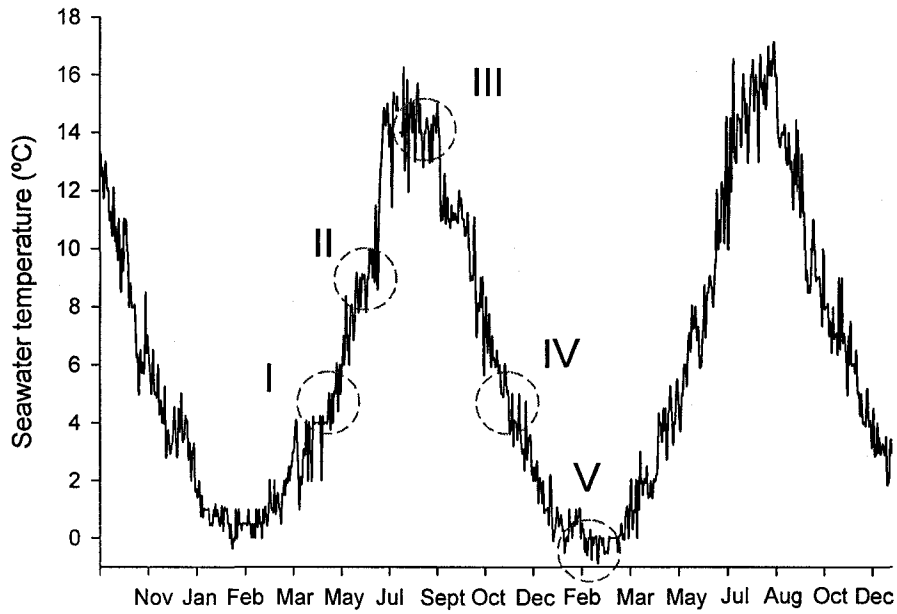


Figure 3.3 – Temperature profile in the seawater system at the Ocean Sciences Centre in 2005/2006. Dashed circles show the approximate times of year when the seawater was at the desired temperatures. Roman numerals indicate the measurement periods as defined in Table 3.1.

For this experiment, ten cunner were randomly chosen from the initial group, and transferred to a separate tank where they were maintained throughout the year.

After being fasted for an appropriate period of time (active *vs.* dormant), individual fish were transferred from their holding tank to the respirometer. Routine metabolic rate and water temperature were measured at the beginning of every hour, for a total of 72 hours. The first 48 hours were determined based on preliminary experiments (see Appendix B), as the time necessary

for the fish to recover from handling and to habituate to the conditions within the respirometer. After the protocol was complete the fish was gently removed from the respirometer, measured for mass and length, and returned to its holding tank. Fish were tagged, by attaching a colored bead to the dorsal fin, under light anaesthesia (0.1g L^{-1} of MS-222, ethyl-*m*-aminobenzoate), after their first measurement to provide individual recognition in later measurements. After the final measurement, all fish were killed by anaesthetic overdose with 0.3g L^{-1} of MS-222 (ethyl-*m*-aminobenzoate) and their sex determined by visual examination of the gonads. Seasonal MRr at each measurement period/temperature was calculated by averaging individual MRr for the last 24 hours inside the respirometer. Similarly, seasonal day and night MRr values were obtained by averaging the day and night MRr(s) of individual fish in the last full day/night cycle. Routine metabolic rate Q_{10} values, between each temperature/season, were calculated using the mean MRr value at each temperature/season.

3.2.3 Acute Temperature Challenges

These experiments were performed during late fall and winter when seawater temperatures were around 5 and 0°C, respectively. The fish used in late fall (~5°C) were still active and at the point where they were beginning to show signs of entering dormancy, while the fish used in winter (at 0°C) had been dormant for at least 2 months.

The acute temperature challenges were performed by decreasing (fish at 5°C, n = 8) or increasing (fish at 0°C, n = 8) the water temperature by 5°C, one degree per hour, and then maintaining the cunner at the final temperature (0 or 5°C) for an additional 3 hours. Individual fish were transferred from their holding tanks to the respirometer and, similarly to the seasonal

experiment, given 48 hours to recover from handling and to habituate to the respirometer. At 9am of the third day, a control measurement of MRr was recorded and the protocol initiated. The temperature drop/increase was achieved by regulating the temperature of the reservoir. Temperature in the reservoir usually dropped/increased within 30 minutes prior to being maintained at that temperature for the remaining 30 minutes. Temperature was constantly monitored and MRr measurements made at the end of every hour throughout the protocol. Water flow rate through the respirometer (Vw) was measured every time an oxygen concentration reading was performed, ranging from 0.4 to 0.6L h^{-1} between trials. After the final measurement, fish were killed by an anaesthetic overdose (0.3g L^{-1} of MS-222, ethyl-*m*-aminobenzoate), measured for mass and length, and their sex determined by visual examination of the gonads. Routine metabolic rate Q_{10} values, between the control value (5 or 0°C) and the remaining temperatures/measurement points, were calculated using the mean MRr value at each temperature/measurement point.

3.2.4 Statistical Analyses

All values are expressed as means ± standard error (SE) of the mean. All statistical analyses were performed using MINITAB™ 13.1 (Minitab Inc) and the General Linear Model, and $P < 0.05$ was used as the level of statistical significance.

To test for significant differences in MRr between seasonal temperatures, a repeated measures two-way ANOVA (main effects temperature/season and time of day) was used. A two-way ANOVA was chosen in order to include the variable time of day, and to test for differences between MRr measured during the day and during the night. This analysis was performed because

time of day (day *vs.* night) appeared to affect MRr, especially at elevated test temperatures (see Appendix B).

To test for differences between MRr during the acute temperature challenges, a repeated measures one-way ANOVA was used. This initial analysis was followed by Dunnett's post-hoc tests in order to check for differences between the control temperature (5 and 0 °C for fall and winter fish, respectively) and the other temperatures/measurement points.

To test for differences between seasonal MRr and MRr obtained during the acute challenges one-way ANOVAs were used.

3.3 Cardiac Function

Cunner cardiac function was assessed through the surgical implantation of a Doppler flow probe on the ventral aorta.

3.3.1Surgery

Fish were initially anaesthetized by immersion in seawater (at approximately seasonal temperature) containing MS-222 at a concentration of 0.15g L^{-1}. The animals were then placed on the operating table, covered with ice, and the gills continuously irrigated with oxygenated seawater (at approximately seasonal temperature) containing 0.05g L^{-1} of anaesthetic.

Implantation of the flow probe was performed as previously described by Brodeur et al. (2001) for rainbow trout, with some modifications. Briefly, the gills and the operculum were retracted using umbilical tape (Baxter Healthcare Corporation, Deerfield, IL) which was passed from a hole behind the 3rd gill arch into the opercular cavity. The 4th gill arch in these fish is fused to the opercular

cavity and therefore the lamellae of the 4th gill arch were pulled back with the help of a cotton swab and a wet Kim Wipe (Kimberly Clark®). The ventral aorta was exposed by a small incision (approximately 5mm in length) in the skin and connective tissue at the base of the 4th gill arch, without disrupting the pericardium, and a silicone cuff-type Doppler flow probe (Iowa Doppler Products, Iowa City, IA, 1.3mm, 20 MHz) was fitted around the vessel (Fig. 3.4). Finally, after verifying the quality of the signal the probe leads were sutured to the skin of the fish using 3-0 silk (American Cyanamid Company, Pearl River, NY) at two locations on the edge of the opercular cavity.

Figure 3.4 – Implantation of a silicone cuff-type Doppler flow probe on the ventral aorta. A) The ventral aorta (↑) is first exposed with a small incision in the skin and connective tissue; B) The flow probe is then fitted around the vessel (1) and the probe leads (2) sutured to the skin.

3.3.2 Recovery from surgery

Recovery from anaesthesia was initiated immediately after surgery by artificially ventilating the fish with aerated, anaesthetic-free seawater (at approximately seasonal temperature). Once

ventilatory activity was re-established, the fish was placed in a 12L flow-through chamber supplied with seawater (at the seasonal temperature of the ocean). The water in the chamber was continuously replaced using a submersible pump (model NK-1, Little Giant Co., USA) from a 135L reservoir filled with temperature controlled air saturated seawater. Water temperature in the reservoir was maintained by a refrigerating/heating circulating bath (model 1150S, VWR International) and titanium heat exchange coils (Fig. 3.5).

The chamber used was opaque and dark in color to minimize visual disturbances. Although fish appeared to fully recover from surgery after 24 hours, a 48 hour period of habituation to the chamber was maintained for consistency with MRr experiments.

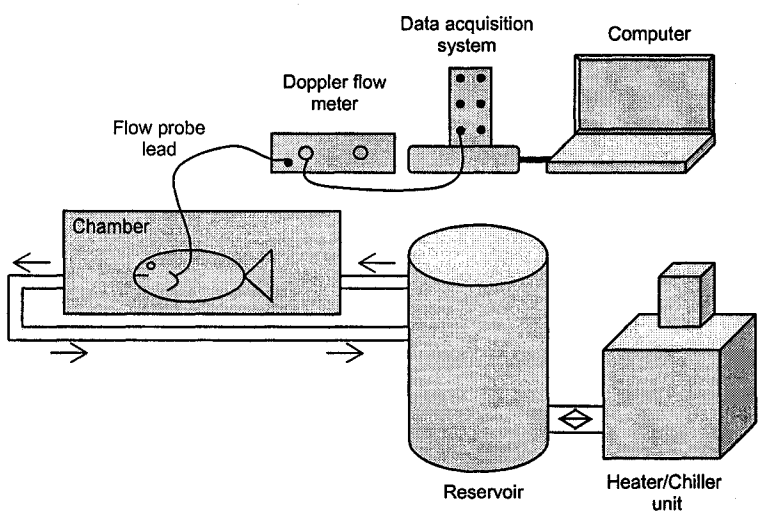


Figure 3.5 – Diagram of the experimental set-up used for the measurement of cunner cardiac function. See text for details.

3.3.3 Data Acquisition and Analysis

Cardiac output (in Volts) was monitored by connecting the Doppler blood flow probe leads to a directional pulsed Doppler flowmeter (Model – 545C-4; Bioengineering, University of Iowa, Iowa City). The flow signal was then amplified and filtered using a Model MP100A-CE data acquisition system (BIOPAC systems Inc., Santa Barbara, CA), and the acquired signals analyzed and stored using AcqKnowledge 3.7.2 (BIOPAC systems Inc., Santa Barbara, CA) installed on a Toshiba laptop computer.

Doppler flow probes provide reliable information on zero flow and relative changes in blood flow. However, in order to estimate absolute blood flow values, the probes must be calibrated. Thus, a careful *in situ* calibration of each flow probe was performed following the procedure outlined in Brodeur et al. (2001). Basically, after the experiment was complete the fish was over anaesthetized with 0.3g L^{-1} of MS-222 (ethyl-*m*-aminobenzoate), measured for mass and length, and carefully placed ventral side up on a surgery table. After exposing the heart, the ventricle was cut in half and carefully cannulated with P50 tubing (Intramedic Clay Adams™, Becton Dickson, Maryland, USA) without disrupting the position the flow probe (Fig. 3.6). Immediately after cannulating the heart, the first gill arch was cut and the heart slowly perfused with a saline solution (0.9% NaCl, i.e. 0.9g/100ml) containing 100 units of heparin per ml. This was done to clear the heart and vasculature of any air bubbles or blood clots that may have resulted from the cannulation procedure. After checking the quality of the signal, the heart was then perfused with diluted (1:4, with 0.9% NaCl) pig's blood at several known flow rates using a peristaltic pump (Masterflex L/S model 7523-20, Cole-Palmer). The blood/saline mix was constantly stirred, and kept on ice

throughout the calibration procedure. After this procedure was complete the fish's sex was determined by visual examination of the gonads.

Values for absolute Q (in ml min^{-1} kg^{-1}) were calculated from the regression equation line between the voltage values obtained during the calibration protocol and the blood flow rate (see Appendix C for a more detailed explanation). Heart rate (f_H, beats min^{-1}) was calculated by measuring the number of systolic peaks during a 30 second interval, while mass specific stroke volume (SV_H, ml kg^{-1}; i.e. volume of blood ejected with each heartbeat), was calculated as:

$SV_H = (Q / f_H) / M$; where M = Mass of fish (kg)

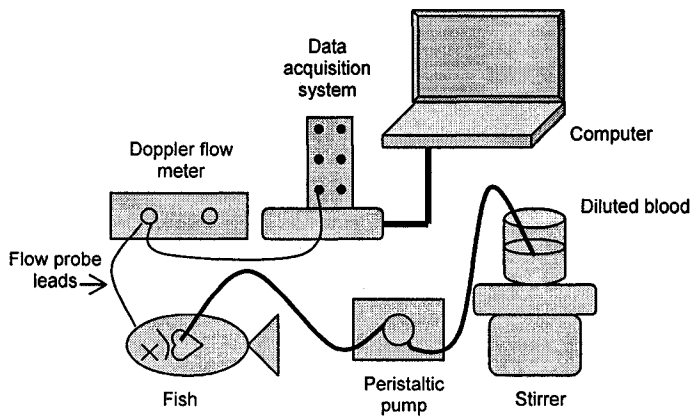


Figure 3.6 – Diagram of set-up used to perform the *in situ* calibration of the Doppler flow probe. See text for details.

3.3.4 Seasonal Effects on Cardiac Function

As in the seasonal MRr experiment, measurements of cunner cardiac function were conducted at several times during the year. These measurement periods corresponding to periods III, IV and V (14, 5 and 0°C, respectively), as shown in Table 3.1 and Fig. 3.3. As previously mentioned, fish at 14°C (late summer) were fully active, while fish at 5°C (late fall) were still active but about to go

into dormancy, and fish at 0°C (winter) had already been dormant for at least 2 months. Temperature and cardiac function were continuously recorded throughout the experiment. However, for the purpose of measuring seasonal cardiac function, at approximately 9am of the third day following the surgery (after approx. 48 hours of recovery), ventral aortic blood flow (Q, in volts) and f_H were measured for 5 minutes; the later by counting the number of systolic peaks in a 30 second period. Due to the terminal nature of the protocol (*in situ* calibration) the seasonal experiment was done using 3 different groups of fish, referred to as the 14, 5 and 0°C cardiac function groups (for mass and length information see Appendix A). Cardiac output, heart rate and stroke volume Q_{10} values, between each temperature/season (at 14 and 5°C or 5 and 0°C), were calculated using the mean values for Q, f_H or SV_H at each temperature/season.

3.3.5 Acute Temperature Challenges

These experiments were performed using the same fish that were used for the seasonal measurements of cardiac function (periods IV, 5°C in fall; and V, 0°C in winter). At 9am of the third day (i.e. after approx. 48h of recovery) the seasonal/control measurement (5 and 0°C for fall and winter fish, respectively) was recorded (see section 3.3.4) and the acute temperature challenge protocol initiated. Similarly to what occurred for the MRr experiment, the acute temperature challenges were performed by decreasing (fish at 5°C, n = 7) or increasing (fish at 0°C, n = 7) the water temperature by 5°C, one degree per hour. Further, the fish were maintained at the final temperature 0 or 5°C, respectively, for an additional 3 hours. The temperature drop/increase was achieved by regulating the temperature of a 135L reservoir, using a refrigerating/heating circulating bath (model 1150S, VWR International). Similar to the MRr experiment, the temperature was

usually decreased/increased within 30 minutes, and was then maintained at that level for an additional 30 minutes. At the end of each hour, ventral aortic blood flow (Q, in volts) and f_H were recorded for five minutes, the latter determined by counting the number of systolic peaks in a 30 second period. After the final measurement, a calibration of the Doppler flow probe was performed (see section 3.3.3). Cardiac output, heart rate and stroke volume Q_{10} values, between the control value (5 or 0°C) and the remaining temperatures/measurement points, were calculated using the mean value of each parameter at each temperature/measurement point.

3.3.6 Statistical Analyses

The measured parameters are reported as means ± standard error (SE) of the mean. All statistical analyses were performed using MINITAB™ 13.1 (Minitab Inc) and the General Linear Model, and $P < 0.05$ was used as the level of statistical significance.

A one-way ANOVA, followed by Tukey's post-hoc tests, was used to determine significant differences in cardiac output (Q), heart rate (f_H) and stroke volume (SV_H) between the three seasonal temperatures.

For the acute temperature challenges, a repeated measures one-way ANOVA was used for each cardiac parameter (Q, f_H and SV_H), followed by post-hoc Dunnett's tests, to check for differences between the values at the control temperature (5 or 0 °C) and subsequent temperatures/measurement points.

Finally, to compare seasonal Q, f_H or SV_H values with those obtained in the acute temperature decrease challenge, one-way ANOVAs were used.

4. Results

4.1 Routine Metabolic Rate

Seasonal Effects on Routine Metabolic Rate

There were dramatic seasonal variations in cunner routine metabolism, with MRr varying from 7.9 ± 0.3 mg O_2 $kg^{-0.83}$ h^{-1} at 0°C to 57.1 ± 2.5 mg O_2 $kg^{-0.83}$ h^{-1} at 14°C (Fig. 4.1A) over the year. From early spring, when the fish come out of dormancy (5°C), to the end of spring/early summer (9°C) there was an increase in (MRr) of approximately 2 fold (from 15.9 ± 0.4 to 31.8 ± 1.3 mg O_2 $kg^{-0.83}$ h^{-1}), with a Q_{10} of 6.3. The Q_{10} value then dropped to 3.1 when temperature increased to 14°C, in late summer. Routine metabolic rate in late fall (5°C), prior to the cunner entering a dormant state, fell by 56% from the MRr recorded at 14°C (Q_{10} = 2.5). This result was surprising however, because: 1) MRr in the fall (5°C) was similar to that measured in the spring/summer (9°C), and significantly higher (~40%) than MRr recorded at 5°C in the spring; and 2) the Q_{10} (based on the data for individual fish) for MRr from 5 to 14°C (4 ± 0.4, spring to summer) was significantly higher (P=0.009) than that measured from 14 to 5°C (2.5 ± 0.3; summer to late fall). These results clearly indicate that environmental cues other than water temperature, or the physiological state of the animal during 'arousal', influence MRr in this species. The last measurement for this experiment was performed during the winter when the seawater temperature was around 0°C. At this temperature MRr was extremely low, and the 69% drop in MRr from 5°C (fall) resulted in an extremely high Q_{10} value (Q_{10} ~10).

From Appendix B, it was clear, especially at the highest temperature (14°C), that there was a diurnal cycle in cunner metabolic rate. Thus, average values for the daytime and nighttime of the 3rd day were calculated at each season (temperature) to see if the diurnal cycle in metabolism

might affect the overall interpretation of results (Fig. 4.1B). Values for the day and night were similar at most temperatures, and even though MRr in the nighttime was 25.6% higher than daytime values in summer (at 14°C), these values were not significantly different (P = 0.148). Further, the overall pattern of change for MRr with season was similar for each time of day.

Metabolic Response to Acute Temperature Challenges

The metabolic response to the acute temperature challenges varied depending on whether the temperature was increased or decreased by 5°C.

During the period when water temperature was being lowered, the cunner was able to regulate MRr relatively close to the control value (5°C; 42.2 ± 8.2 mg O_2 $kg^{-0.83}$ h^{-1}). However, after the first hour at 0°C MRr significantly (P = 0.045) dropped (38%) from control value, and after 2 hours at 0°C MRr decreased even further, showing a total drop of 64% from the control (Time 0) value; the change in MRr between control (5°C) and 2 - 3 hours at 0°C representing a Q_{10} of approximately 8 (Fig. 4.2).

In contrast to the decreasing temperature challenge, MRr paralleled the increase in temperature, and was significantly elevated as compared to the control (0°C) value (11.5 ± 1.4 mg O_2 kg^{-1} h^{-1}) by the time water temperature reached 3°C (Fig. 4.3). Further, it appears that MRr continued to increase after the water temperature reached 5°C, being maximal after 3 hours at that temperature (32.6 ± 2.9 mg O_2 kg^{-1} h^{-1}). This suggests that 3 hours at the final temperature (5°C) was not sufficient for MRr to reach a steady state, and thus, that the measurement period following an acute temperature increase be extended by several hours in future studies.

Figure 4.1 – The effect of season on the routine metabolic rate (MRr) of cunner (n = 7; mass = 224 ± 17g; length = 24 ± 0.5cm). A) Values are the average of the last 24 hours inside the respirometer. B) Day and night averages. A repeated measures two-way (season and day/night) ANOVA was used to test for significant differences. Values without a letter in common are significantly different ($P < 0.05$); The Q_{10} values presented were calculated using the temperatures indicated by the arrows.

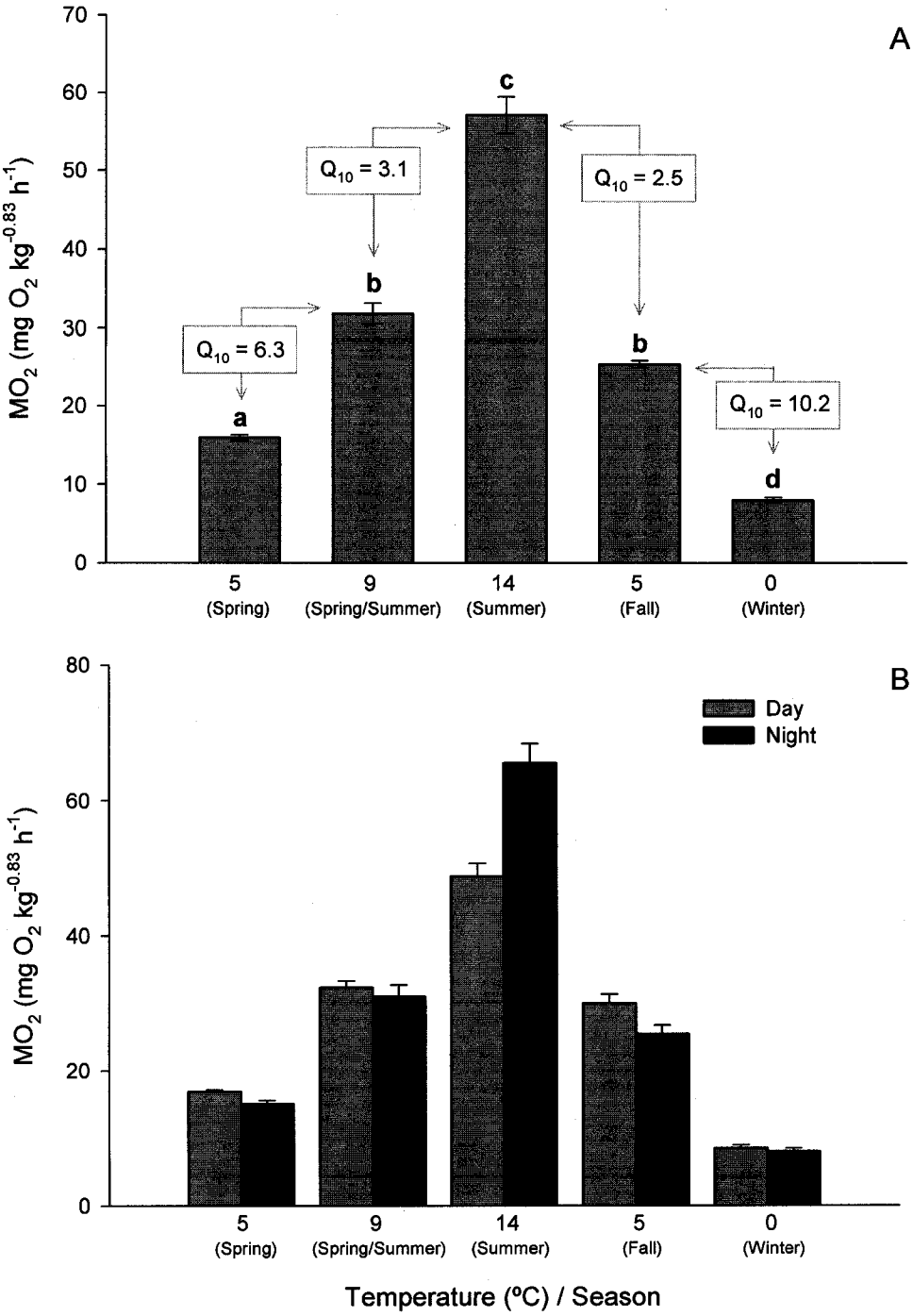

A
70
60
50
40
30
20
10
0
MO2 (mg O2 kg-0.83 h-1)
Q10 = 6.3
Q10 = 3.1
Q10 = 2.5
Q10 = 10.2
a
b
c
b
d
5 (Spring)
9 (Spring/Summer)
14 (Summer)
5 (Fall)
0 (Winter)
B
80
60
40
20
0
Day
Night
MO2 (mg O2 kg-0.83 h-1)
5 (Spring)
9 (Spring/Summer)
14 (Summer)
5 (Fall)
0 (Winter)
Temperature (ºC) / Season

Figure 4.2 - Cunner routine metabolic rate (MRr_r) during an acute temperature decrease challenge (n = 8; mass = 190 ± 18.4g; length = 24 ± 0.7cm). A repeated measures one-way ANOVA, followed by a Dunnett's post-hoc test ($P < 0.05$), was used to test for significant differences (*) as compared with the control value (measurement at time 0h). The Q_{10} values presented were all calculated relative to the control value.

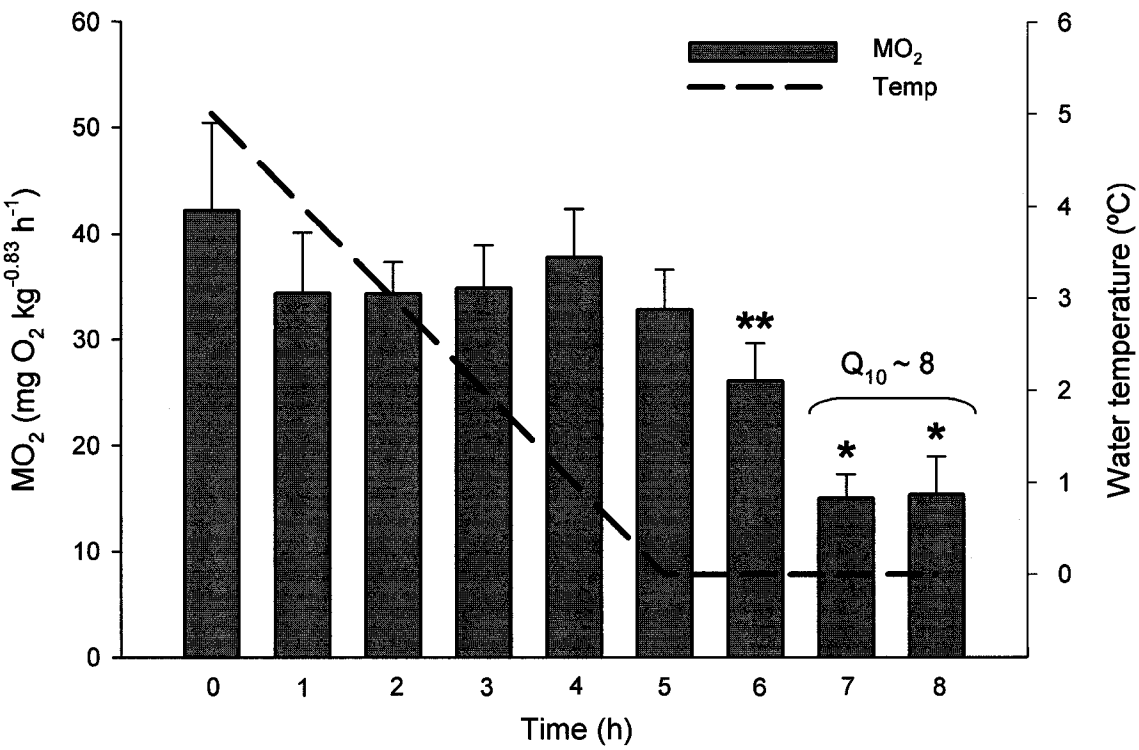
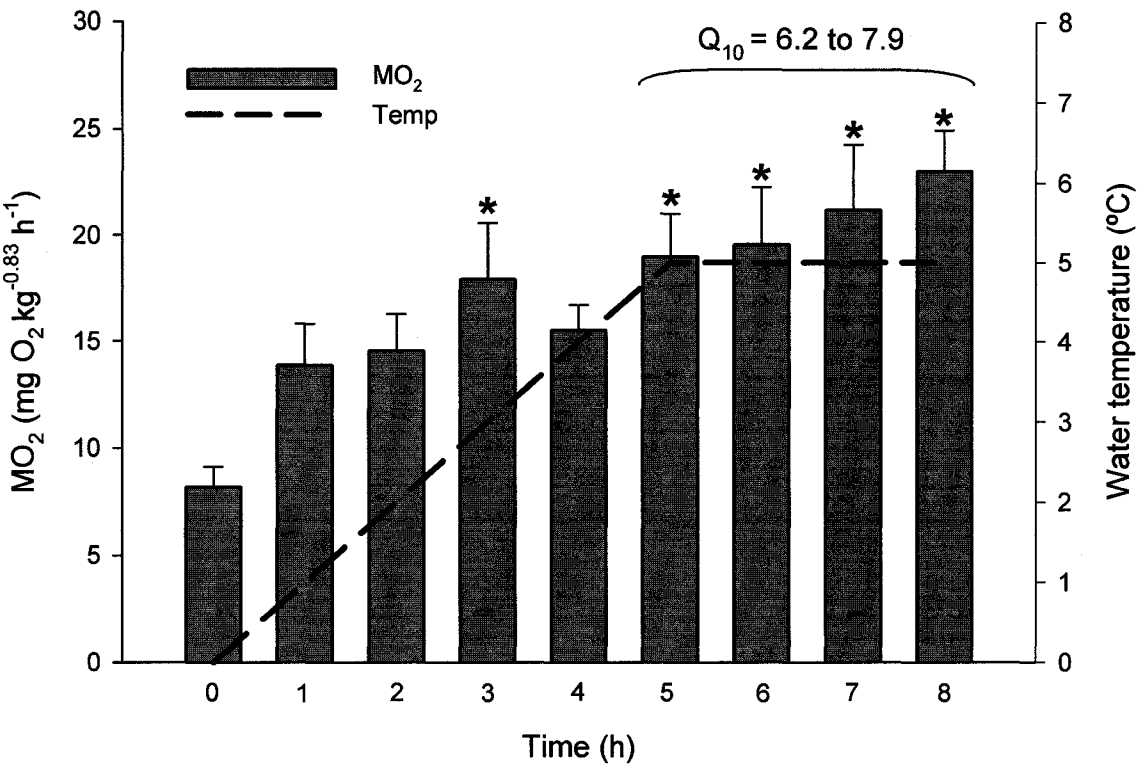

MO2
Temp
MO2 (mg O2 kg-0.83 h-1)
Water temperature (°C)
Time (h)
Q10 ~ 8
**
*
*
0
1
2
3
4
5
6
7
8
10
20
30
40
50
60

Figure 4.3 - Cunner routine metabolic rate (MRr) during an acute temperature increase challenge (n = 8; mass = 138 ±13g; length = 21 ± 0.5cm). A repeated measures one-way ANOVA, followed by a Dunnett's post-hoc test ($P < 0.05$), was used to test for significant differences (*) from the control value (measurement at time 0h). The Q_{10} values presented were all calculated relative to the control value.


Q_{10} = 6.2 to 7.9
MO_2
Temp
MO_2 (mg O_2 $kg^{-0.83}$ h^{-1})
Water temperature (°C)
Time (h)
0
1
2
3
4
5
6
7
8
*

Seasonal vs. Acute Temperature Responses

When comparing MRr values obtained in the fall (5°C), winter (at 0°C) and spring (at 5°C), with those obtained when fish were challenged with an acute decrease (5 to 0°C, Figure 4.4) or increase (0 to 5°C, Figure 4.5) in temperature, respectively, we see that there was only one difference between the seasonal and acute challenge at equivalent temperatures. The value recorded after 3 hours at 5°C was significantly higher (by 33.6%) as compared with the seasonal 5°C value (spring, see figure 4.5). However, there were substantial differences in the time-course required for the two acutely challenged groups to reach seasonal values, and in the relation between Q_{10} values obtained acutely *vs.* seasonally. For fish acutely challenged with a temperature decrease, 2 hours at 0°C was required for cunner to decrease their MRr to values similar to what was obtained seasonally (i.e. in winter). Further, the Q_{10} values (at 2 and 3 hours at 0°C) for fish acutely exposed to the temperature decrease were lower than recorded in the seasonal study (8 *vs.* 10.5). In contrast, the MRr of fish exposed to the acute temperature increase was similar to that recorded in the seasonal experiment as soon as they reached 5°C. However, the Q_{10} values for the acutely challenged fish were greater than that recorded seasonally (6.2 - 7.9 *vs.* 4.3).

4.2 Cardiac Function

Seasonal Effects on Cardiac Function

The effect of season on cunner cardiac function (Fig. 4.6) was only examined in early September (summer), November (fall) and February (winter), at 14, 5 and 0°C respectively. In the summer, values for cardiac output (Q), heart rate (f_H) and stroke volume (SV_H) were 29.0 ± 3.9 ml min^{-1} kg^{-1}, 45.6 ± 2.7 beats min^{-1}, and 0.64 ± 0.08 ml kg^{-1}, respectively. Both Q and f_H (Fig. 4.6 A and B)

showed expected Q_{10} values between 14 and 5°C (Q_{10} = 2.8 and 2.5, respectively). In contrast, the Q_{10} values between 5 and 0°C were 7.9 and 5 for Q and f_H, these values representing decreases of 63 and 54%, respectively. There was no significant difference in the 3 SV_H values (range 0.47 to 0.64 ml kg^{-1}), and the Q_{10} values between 14 and 5°C (Q_{10} = 1.1), and 5 and 0°C (Q_{10} = 1.6). These data demonstrate that cunner stroke volume is insensitive to changes in season/acclimation temperature.

When comparing Q and MRr over the 3 measurement periods (Fig. 4.1A *vs.* 4.6A) it is apparent that the effect of decreasing day length/acclimation temperature on the two parameters is similar. The only difference being a smaller Q_{10} value between fall and winter for cardiac output (7.9 *vs.* 10.2).

Cardiac Response to Acute Temperature Challenges

Consistent with the experiment that examined changes in cardiac function across seasons, SV_H was insensitive to acute changes in temperature (Figs. 4.7C and 4.8C). In general, the pattern of changes in Q and f_H with increases and decreases in temperature were similar. That is, these two parameters were linearly related to temperature, and values at the final temperature (0 or 5°C) were constant for the 3h period of exposure (Figs. 4.7A and B, and 4.8A and B). However, there was a clear difference in the magnitude of the change, with Q_{10} values for the temperature decrease challenge (5 to 0°C) ranging from 2.0 to 2.9 as compared with 4.4 to 6.3 for the temperature increase experiment (0 to 5°C).

Figure 4.4 - Comparison of routine metabolic rate (MRr) obtained in the seasonal study (fall 5 °C, winter 0 °C) and in the acute temperature decrease challenge. One-way ANOVA was used to test for differences between seasonal and acute MRr values at equal temperatures; (*) indicates significant differences ($P < 0.05$). The Q_{10} values presented were calculated between the temperatures indicated by the arrows.

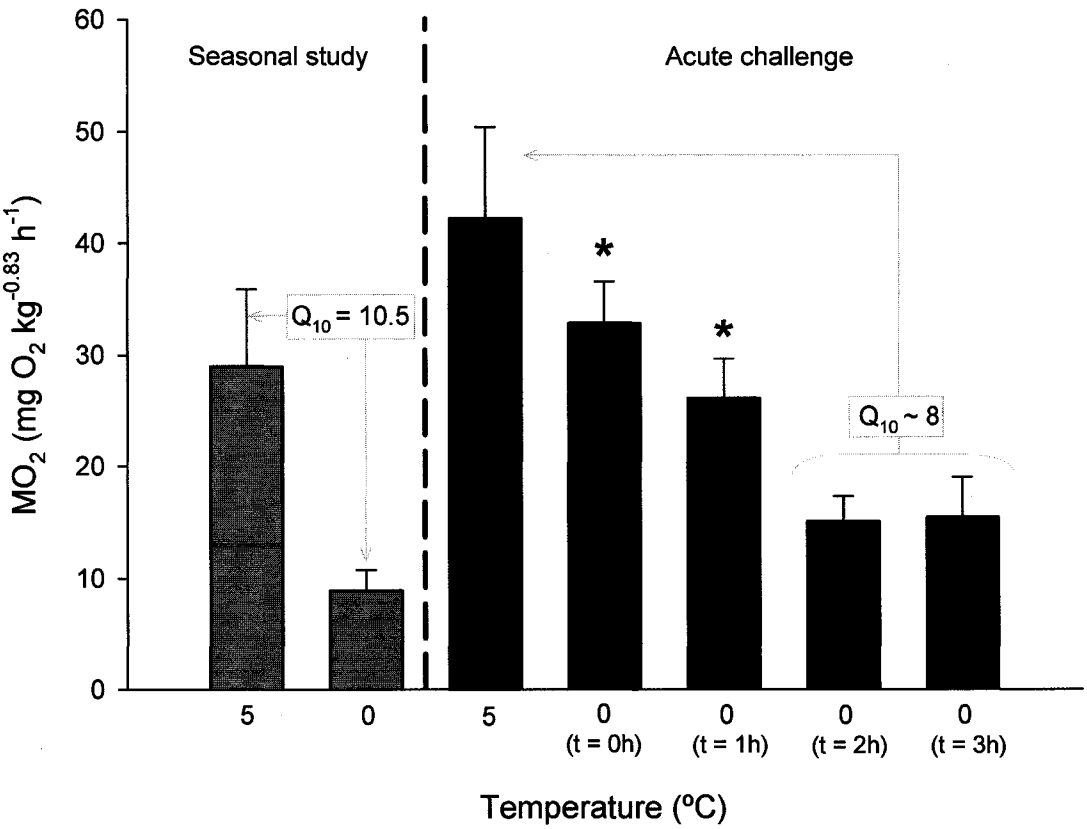

Seasonal study
Acute challenge
Q_{10} = 10.5
Q_{10} ~ 8
*
*
MO_2 (mg O_2 $kg^{-0.83}$ h^{-1})
60
50
40
30
20
10
0
5
0
5
0 (t = 0h)
0 (t = 1h)
0 (t = 2h)
0 (t = 3h)
Temperature (ºC)

Figure 4.5 - Comparison of routine metabolic rate (MRr) obtained in the seasonal study (winter 0ºC, spring 5ºC) and in the acute temperature increase challenge. One-way ANOVAs were used to test for differences between seasonal and acute MRr values at equal temperatures; (*) indicates significant differences ($P < 0.05$). The Q_{10} values presented were calculated between the temperatures indicated by the arrows.

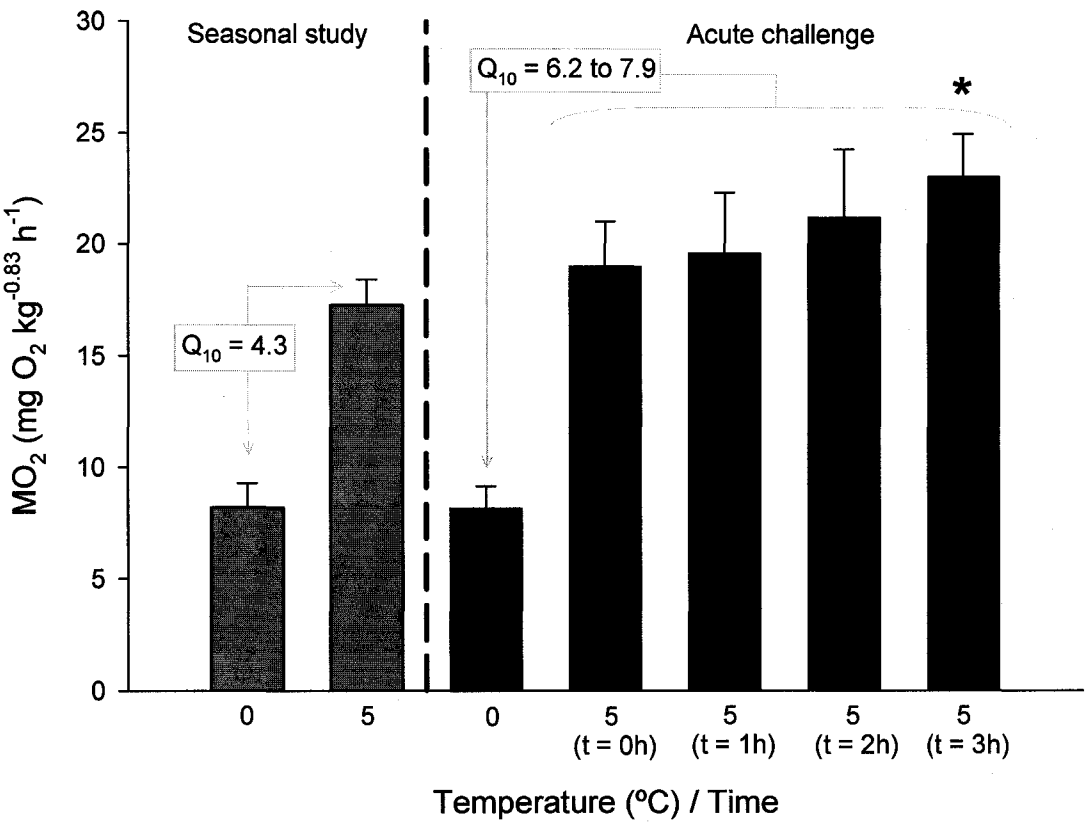

Seasonal study
Acute challenge
Q_{10} = 6.2 to 7.9
Q_{10} = 4.3
*
MO_2 (mg O_2 $kg^{-0.83}$ h^{-1})
30
25
20
15
10
5
0
0
5
0
5 (t = 0h)
5 (t = 1h)
5 (t = 2h)
5 (t = 3h)
Temperature (°C) / Time

Figure 4.6 – The effect of season/temperature on cardiac function of cunner; A) Cardiac output (Q), B) heart rate (f_H) and C) stroke volume (SV_H). Group at 14°C (n = 7; mass = 271 ± 14g; length = 27 ± 0.3cm), group at 5°C (n = 7; mass = 220 ± 21g; length = 23 ± 0.9cm) and group at 0°C (n = 7; mass = 227 ± 6g; length = 25 ± 0.6cm). Different groups of fish were used at each season/temperature due to the terminal nature of the protocol. A repeated measures one-way ANOVA, followed by a Tukey's post-hoc test, was used to test for significant differences. Values without a letter in common indicate a statistical difference ($P < 0.05$). The Q_{10} values were calculated between the temperatures connected by the arrows.

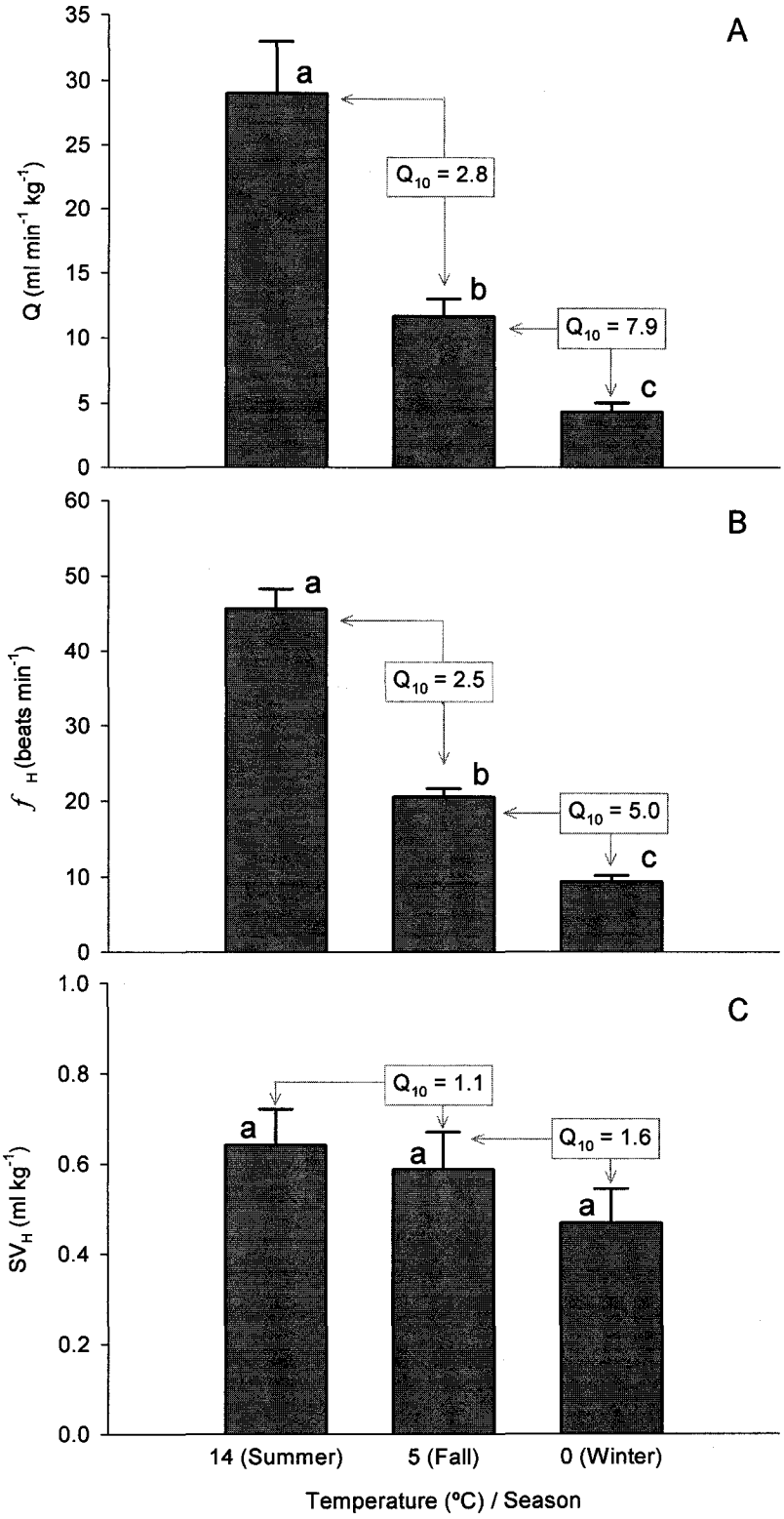

A
B
C
Q (ml min-1 kg-1)
fH (beats min-1)
SVH (ml kg-1)
Q10 = 2.8
Q10 = 7.9
Q10 = 2.5
Q10 = 5.0
Q10 = 1.1
Q10 = 1.6
a
b
c
14 (Summer)
5 (Fall)
0 (Winter)
Temperature (°C) / Season

Figure 4.7 – Cunner cardiac function, A) cardiac output (Q), B) heart rate (f_H) and C) stroke volume (SV_H) during an acute temperature decrease from 5 to 0°C; (n = 7; mass = 220 ± 21g; length = 23 ± 0.9cm). Temperature inside the respirometer is shown by the dashed line. A repeated measures one-way ANOVA, followed by a Dunnett's post-hoc test ($P < 0.05$), was used to test for significant differences (*) from the control value (measurement at time 0h). The Q_{10} values presented were all calculated relative to the control value.

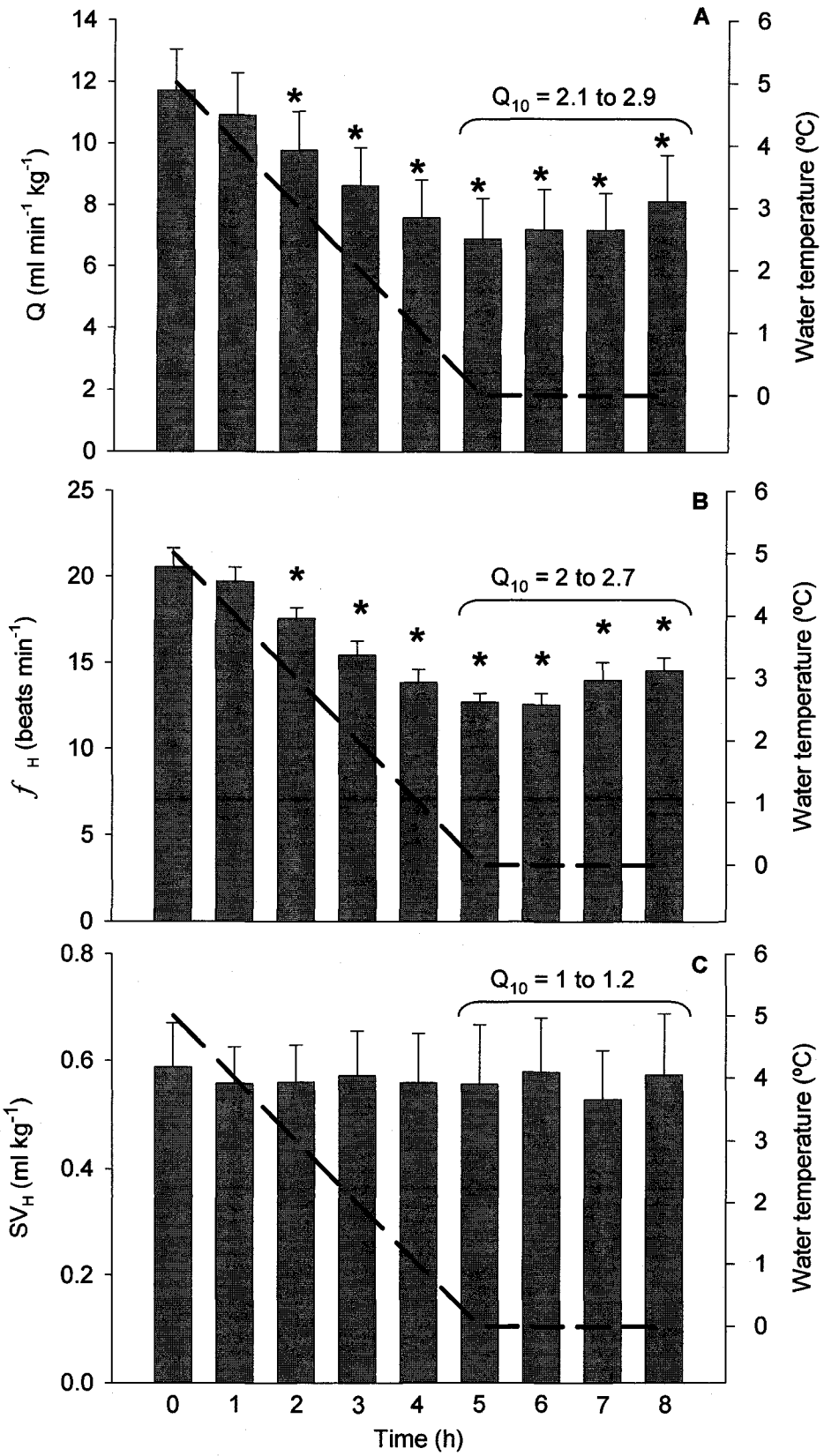
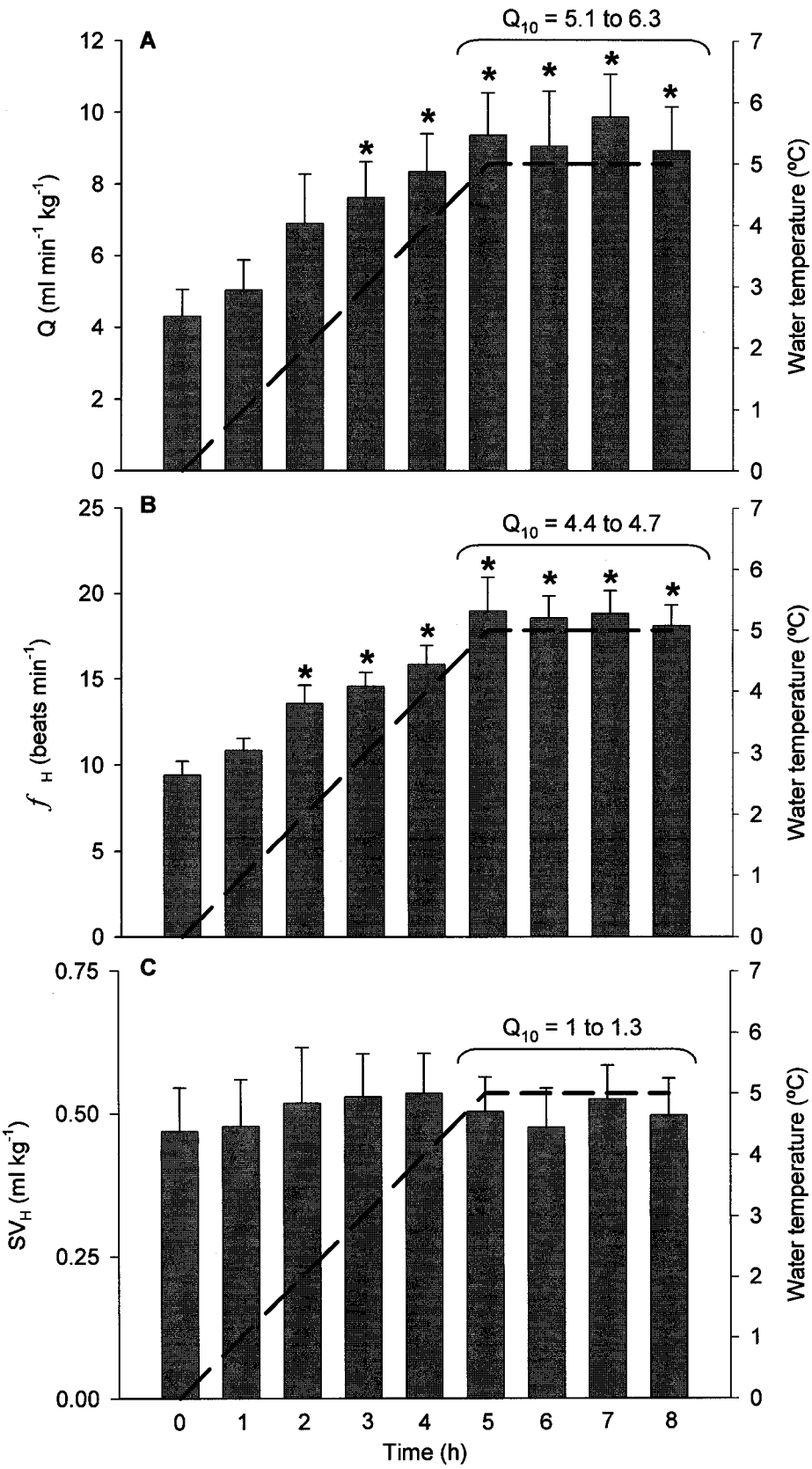

A
B
C
Q (ml min-1 kg-1)
fH (beats min-1)
SVH (ml kg-1)
Water temperature (°C)
Q10 = 2.1 to 2.9
Q10 = 2 to 2.7
Q10 = 1 to 1.2
Time (h)

Figure 4.8 – Cunner cardiac function, A) cardiac output (Q), B) heart rate (f_H) and C) stroke volume (SV_H) during an acute temperature increase from 0 to 5°C; (n = 7; mass = 227 ± 6g; length = 25 ± 0.6cm). Temperature inside the respirometer is shown by the dashed line. A repeated measures one-way ANOVA, followed by a Dunnett's post-hoc test ($P < 0.05$), was used to test for significant differences (*) from the control value (measurement at time 0h). The Q_{10} values presented were all calculated relative to the control value.

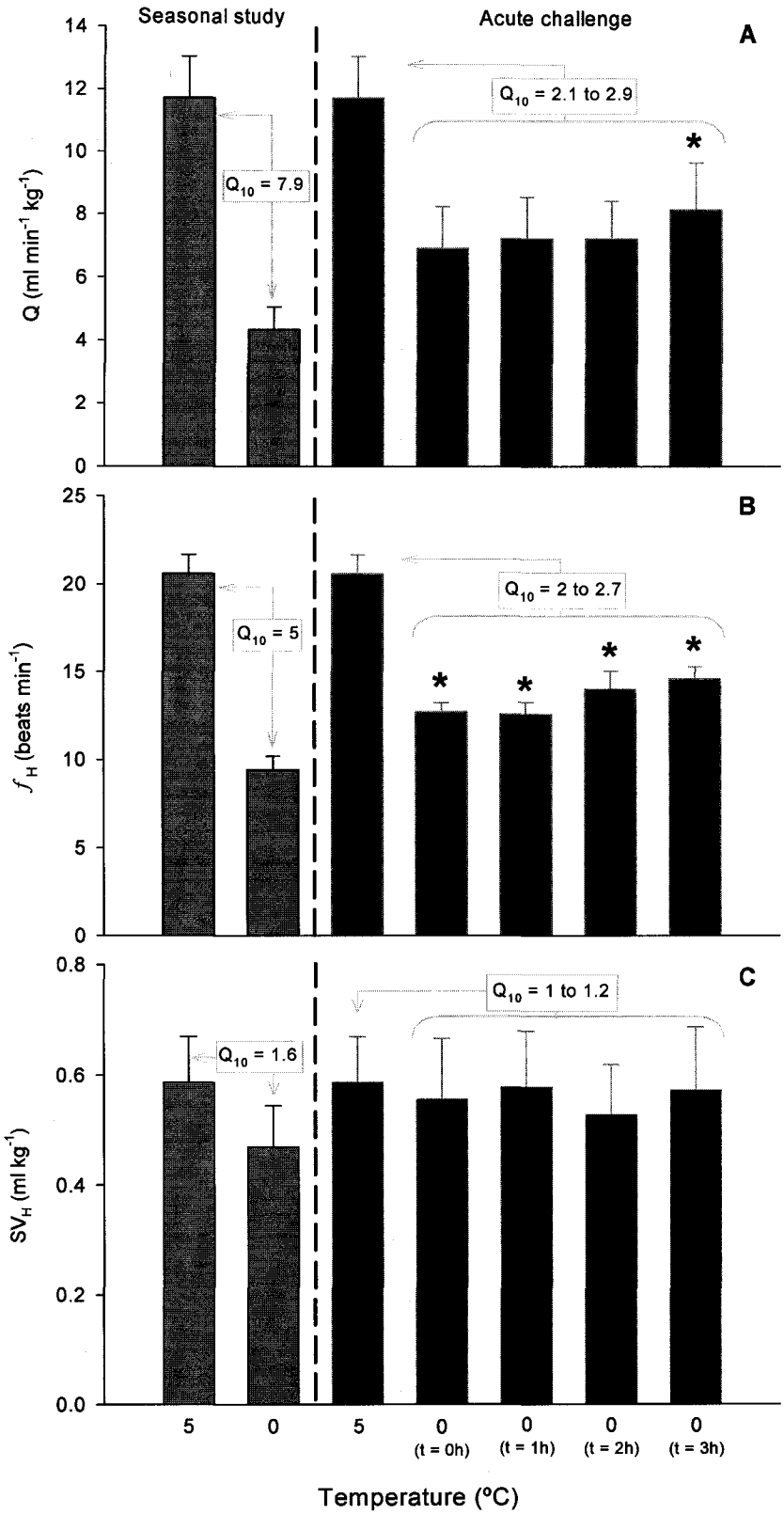

A
B
C
Q_{10} = 5.1 to 6.3
Q_{10} = 4.4 to 4.7
Q_{10} = 1 to 1.3
Q (ml min^{-1} kg^{-1})
f_H (beats min^{-1})
SV_H (ml kg^{-1})
Water temperature (ºC)
Time (h)

The responses of MRr (Fig. 4.3) and Q (4.8A) to an acute temperature increase were very similar, with the exception of slightly lower Q_{10} values for Q (5.1 – 6.3 as compared with 6.2 – 7.9). In contrast, there were clear differences between the responses of these two parameters to a 5°C temperature decrease (Figs. 4.2 and 4.7A). For example: 1) MO_2 was constant when temperature was reduced from 5 to 0°C, whereas Q fell in proportion to the change in temperature, and 2) the Q_{10} values for the 5 - 0°C change were 4 fold greater for MO_2 (approx. 8) than for Q (2.1 – 2.9). These results show that there is a decoupling between MRr and Q responses to an acute drop in temperature (from 5 to 0°C).

Seasonal vs. Acute Temperature Responses

When comparing cunner cardiac function during the seasonal study (i.e. fall, 5°C *vs.* winter, 0°C) (Fig. 4.6), with that measured when this species was challenged with an acute temperature decrease from 5 to 0°C (Fig. 4.7), it is apparent that the seasonal effect on cardiac output and heart rate was much greater. This conclusion based on: 1) the Q_{10} values for Q and f_H being approximately 2 fold and 4 fold greater during the seasonal experiment, respectively; and 2) the observation that these parameters were substantially or significantly higher in the 1 to 3 hours after acute exposure to 0°C, as compared with the winter acclimated cunner. Not surprisingly, no difference was found between the seasonal and acute effects of a temperature on SV_H (Fig. 4.9C).

Figure 4.9 - Comparison of the response of cunner cardiac function to a seasonal decrease in water temperature (fall 5°C, winter 0 °C; represented by the grey bars) with that shown when this species was acutely exposed to a temperature decrease from 5 to 0°C in the fall (black bars). A) Cardiac output (Q); B) heart rate (f_H); and C) stroke volume (SV_H). One-way ANOVAs were used to test for differences between seasonal and acute values at equal temperatures; (*) indicates significant differences ($P < 0.05$). The Q_{10} values presented were calculated between the temperatures indicated by the arrows.


Seasonal study
Acute challenge
A
B
C
Q (ml min-1 kg-1)
fH (beats min-1)
SVH (ml kg-1)
Q10 = 7.9
Q10 = 2.1 to 2.9
Q10 = 5
Q10 = 2 to 2.7
Q10 = 1.6
Q10 = 1 to 1.2
*
5
0
5
0 (t = 0h)
0 (t = 1h)
0 (t = 2h)
0 (t = 3h)
Temperature (°C)

5. DISCUSSION

5.1 Routine Metabolic Rate

The metabolic rate of cunner at 0°C in the seasonal study was 7.9 ± 0.3 mg O_2 $kg^{-0.83}$ h^{-1}, a value not unexpected based on previous reports that this species becomes dormant in winter (Green and Farwell, 1971). Interestingly, however, the metabolic rate of cunner at 5°C and above (15.9 ± 0.4 to 25.3 ± 0.5 mg O_2 $kg^{-0.83}$ h^{-1} at 5°C; 31.8 ± 1.3 mg O_2 $kg^{-0.83}$ h^{-1} at 9°C and 57.1 ± 2.4 mg O_2 $kg^{-0.83}$ h^{-1} at 14°C) also appears to be lower than that measured for most other fish species at comparable temperatures. For example, although the distribution and habitat of cunner and juvenile cod (both Atlantic cod *Gadus morhua* and Greenland cod *Gadus ogac*) overlap in the North Atlantic, Greenland cod at 4°C (Bushnell et al., 1994) and 8°C (Corkum and Gamperl, unpublished) have routine metabolic rates of 56.3 ± 2.8 and 95.5 ± 6.3 mg O_2 $kg^{-0.83}$ h^{-1}, respectively, and values for the standard metabolic rate of Atlantic cod at 5, 10 and 15 °C are 35.5 ± 4.8, 57 ± 10.9 and 78 ± 9.2 mg O_2 $kg^{-0.83}$ h^{-1}, respectively (Schurmann and Steffensen, 1997). Further, Costa and Gamperl (unpublished) report that, at 8°C, cunner had the lowest routine metabolic rate of the 5 North Atlantic species that they examined, and based on the relationship between metabolic rate and temperature for temperate and tropical fish derived by Steffensen (2002), I estimate that the standard metabolic rate of the cunner should be approximately 50 mg O_2 $kg^{-0.8}$ h^{-1}at 5°C; 75 mg O_2 $kg^{-0.8}$ h^{-1} at 10°C and 110 mg O_2 $kg^{-0.8}$ h^{-1}at 15°C. Collectively, these results suggest that teleost species capable of regulated metabolic depression at cold temperatures have lower metabolic rates than other teleosts at all temperatures. This conclusion is supported by the fact that the MO_2 of cunner at 5 and 14°C was similar to that reported for goldsinnny wrasse at 6°C (~20.5 mg O_2 $kg^{-0.83}$ h^{-1}) and the largemouth bass at 13°C (~42.9 mg O_2

$kg^{-0.77}$ h^{-1}), both species reported to exhibit winter dormancy (Crawshaw et al., 1982; Sayer and Davenport, 1996).

The temperature effect (Q_{10}) on metabolism, in which a change of 10°C usually causes the rate of metabolism to increase or decrease by 2 to 3 fold, has been widely recognized in poikilothermic vertebrates (Rome et al., 1992; Guppy and Withers, 1999). This effect has also been seen in the majority of teleosts (Holeton, 1974) from tropical to polar species (Holeton, 1974, Steffensen, 2002, Steffensen, 2005), at least within a species' natural thermal range (Peck, 2002). In the present study, this magnitude of temperature effect was also observed for cunner routine metabolic rate (MRr) between 9 and 14°C (late spring and summer, Q_{10} = 3.1), as well as when temperature decreased from 14 to 5°C (in the fall, Q_{10} = 2.5). This result is consistent with Curran (1992) who reported a relatively low Q_{10} (Q_{10} = 2.4) in the higher temperature range of her study (between 12 and 22°C) for cunner from Woods Hole, and with data for the goldsinny wrasse (*Ctenolabrus rupestris*), also a member from the Labridae, which showed a Q_{10} of 2.71 between 10 and 8°C (Sayer and Davenport, 1996). However, it is in contrast with data for the American eel (*Anguilla rostrata*), a species that also exhibits winter dormancy. The eels showed some degree of metabolic compensation between 10 and 20°C, with a Q_{10} between 1 and 2 (Walsh et al., 1983).

A decrease in ocean temperature from 5 to 0°C (late fall to winter) caused a 69% decrease in cunner MRr, to approximately 14% of values obtained at 14°C. This abrupt drop in MRr, associated with a very high Q_{10} (~10) between 5 and 0°C, indicates that the depression of cunner MRr was associated with an active downregulation of metabolism, and not solely due to a temperature effect. High Q_{10} values and pronounced metabolic depression have also been observed in other studies. As previously mentioned, both the goldsinny wrasse and the American eel exhibit winter

dormancy, and show high Q_{10}s (≥ 4) when water temperature drops below 6 and 10 °C, respectively (Walsh et al., 1983; Sayer and Davenport, 1996). Further, Curran (1992) observed a high Q_{10} (from 5.5 to 8.9) in a Woods Hole cunner population when seawater temperature fell from 12 to 6.4°C. This latter information suggests that cunner from Woods Hole depress metabolism when the temperature falls below 12°C, or at least somewhere between 12 and 6.4°C, a considerably higher temperature than observed in the present study. This may not be surprising, since the seasonal thermal range that the Woods Hole population is exposed to is considerably warmer (2.5 to 22°C) than for the Newfoundland population (-1 to 14°C ± 1°C).

Since it is generally accepted that the mechanisms responsible for metabolic depression are at the cellular level, it is expected that the downregulation of cellular processes will precede and accompany reductions in whole-animal metabolism. In mammals, 80% of standard metabolic rate is due to mitochondrial oxygen consumption (Rolfe and Brown, 1997), and protein synthesis is a major consumer of ATP produced by oxidative phosphorylation, and thus of the animal's total energy expenditure. For example, it has been estimated that protein synthesis accounts for approximately 20 to 30% of an animal's standard metabolic rate (Brown, 1992; Guppy et al., 1994; Rolfe and Brown; 1997). Lewis and Driedzic (2007) measured *in vivo* protein synthesis rates in cunner from Newfoundland, and found that when the seawater temperature falls from 8 to 4°C these fish show a pronounced depression in rates of protein synthesis; liver, brain, heart, and gill by 55-66%, and below detectable levels in white muscle (Q_{10} ranging from 6.7 to 21). As Lewis and Driedzic (2007) state, it seems that the depression of protein synthesis in cunner is an intrinsic response that precedes the whole-animal metabolic downregulation observed in this species. Interestingly, the only tissue that further depresses protein synthesis when temperature falls to 0°C

is the brain, and according to Lewis and Driedzic (2007) this brings total brain protein synthesis rates to levels between those for anoxic turtles, that exist in comatose state, and the crucian carp which remains active during anoxia. This is interesting because, when the seawater temperature is around 4 - 5°C (in the fall), cunner are still quite active and responsive, although already undergoing a pronounced depression of protein synthesis (e.g. muscle). On the contrary, although these fish are unresponsive and lethargic at 0°C (to the point that they can be easily handled with no need for nets), when returned to their holding tank they promptly swim to a corner and try to hide. Thus, it seems that although the entrance of cunner into dormancy is initiated at the cellular level when the water temperature is between 8 and 4°C, and followed by a pronounced whole-body depression with further reductions in brain cellular function, this species is still capable of some activity while dormant. This may be related to the need to avoid predators, even at temperatures approaching 0°C.

There was a noticeable seasonal effect on cunner metabolism, as MRr at 5°C in the spring was approximately 40% lower than the MRr at 5°C in the fall. This finding is consistent with observations on fish in the respirometer at these temperatures. Fish in late spring were clearly more lethargic than in the fall at the same temperature (5°C). Thus, it seems that cunner are slow to come out of their dormant state. On the other hand, a high Q_{10} (6.3) was found between 5 and 9°C (spring/summer) suggesting that even though they are slow to come out of their metabolically depressed state, they subsequently undergo an active hyperactivation of routine metabolism. Lewis and Driedzic (2007) also found a hyperactivation of protein synthesis in white muscle (from 0 to 4°C), followed by liver and heart, once temperature went back up to 8°C. This initial slow arousal from dormancy might be a consequence of the 6 months of inactivity, without feeding, and

therefore of a poor nutritional status. The subsequent active (Q_{10} = 6.3) increase in whole-animal metabolism, preceded and accompanied by hyperactivation of protein synthesis in several tissues (Lewis and Driedzic, 2007), may be associated with compensatory growth after such long periods of inactivity/food deprivation. Support for the idea that Newfoundland cunner experience compensatory growth in the spring can be found by comparing growth rates between populations. For example, even though cunners in Newfoundland stay dormant for a longer period of time than more southern populations, their annual growth rates remain very similar (Chiasson, 1995).

The acute temperature challenges show some exciting results. First, and contrary to the seasonal study, cunner challenged with an acute temperature decrease show some degree of thermal compensation. For example, they initially maintain MRr constant, and only reduced MRr after water temperature had been at 0°C for 1hour. Second, they were able to depress metabolism to values similar to the seasonal study within 2 hours at 0°C ($Q_{10} \sim 8$). This suggests that, cunners in the fall are able to rapidly depress MRr when acutely challenged with a temperature decrease, and do not require a long period of decreasing temperature and/or photoperiod to successfully downregulate their metabolism. Third, it appears that the metabolism of dormant cunner is not constrained, and that they can rapidly increase MRr when exposed to an acute temperature increase. In fact these results show that if acutely challenged with a 5°C increase, dormant cunner are able to rapidly increase MRr to values higher than observed with seasonal acclimation to 5°C. However, this apparent disparity between seasonal and acute temperature challenges might also be due to the nutritional status of the fish at the time the experiments were performed. In the seasonal study, the fish at 5°C (spring) had spent 5-6 months in dormancy, where feeding was very little or absent. On the other hand, cunners used in the acute temperature challenge were only

dormant for approximately 2 months before the experiments were performed (February). The nutritional status of the 2 groups was probably very different, and this likely affected the amount and type of circulating energy substrates. Starvation experiments using 4°C (dormant) Woods Hole cunner showed that whole-body and liver glycogen, lipid and protein content decreased by 40 to 60% from January to March (Curran, 1992). Further, Alkanani et al (2005) report that after 10 weeks of starvation (at 8°C), Atlantic cod (*Gadus morhua*) showed pronounced decreases in circulating energy substrates (e.g. 33 and 84% decrease in plasma protein and total lipid, respectively). Therefore, it is likely that the fish used in the acute challenges had a better nutritional condition than the seasonal group, and that this allowed them to attain a higher metabolic rate. This is an interesting hypothesis, and further experiments are planned to examine the influence of nutritional status on the degree of metabolic depression during dormancy, and the temperature/time of year when active metabolic depression is initiated.

Overall, these data demonstrate that the cunner possesses a high degree of plasticity with regard to the regulation of MRr, since not only is it able to rapidly lower its MRr with decreasing temperatures but it also able to rapidly increase it when exposed to an acute temperature increase. This plasticity in MRr regulation may not only enable cunner to rapidly react to changing environmental temperatures, but also to avoid predators when dormant at low temperatures, since as previously mentioned, even when dormant, if seriously disturbed, these fish can show some degree of activity.

5.2 Cardiac Function

As previously mentioned, temperature is one of the most important environmental factors affecting the physiology of ectothermic animals, this includes their cardiovascular function (Gamperl and Farrell, 2004; Axelsson, 2005). Cardiac output (Q) is the product of heart rate (f_H) and stroke volume (SV_H), and is tightly coupled with metabolism (Webber et al., 1998; Gollock, et al., 2006) and normally regulated according the needs of an animal (Olson and Farrell, 2006). Resting heart rate is usually set by a combination of pacemaker cell activity, and adrenergic (neural and hormonal) and cholinergic stimulation (Lowe et al., 2005; Olson and Farrell, 2006). Temperature directly affects the intrinsic pacemaker rate (Lillywhite, et al., 1999), and in most fishes the Q_{10} for f_H ranges from 2-3, if temperature remains within the species' normal thermal range (Olson and Farrell, 2006). However, non-polar fish that remain active at low temperatures often show thermal compensation that results from an increase in ventricular mass. This larger ventricle allows for an elevated resting SV_H, and thus the maintenance of Q (Q_{10} ~1) despite low heart rates (Olson and Farrell, 2006).

In contrast to metabolic rate, cardiac function in the cunner appears to be very comparable to that measured in other teleost species at similar temperatures. At 14°C, cunner had resting values for Q and f_H that were similar to those measured in Atlantic cod (*Gadus morhua*) at 14-15°C (~29 ml min^{-1} kg^{-1} and ~49 beats min^{-1}; Gollock et al., 2006), and f_H values comparable to the largescale sucker (*Catostomus macrocheilus*) at 16°C (~40 beats min^{-1}; Kolok et al., 1993). At 5°C, cunner Q and f_H were similar to both winter flounder (*Pseudopleuronectes americanus*) at 4°C (9.8 ± 1.0 ml min^{-1} kg^{-1} and 20.5 ± 1.6 beats min^{-1}, respectively; Joaquim et al., 2004) and largescale sucker at 5°C (~10 ml min^{-1} kg^{-1} and ~22 beats min^{-1}, respectively; Kolok, et al., 1993). Finally, at 0°C,

cunner f_H was similar to that of Antarctic species such as *Pagothenia bernacchii* (10.5 beats min^{-1}) and *Pagothenia borchgrevinki* (11.3 beats min^{-1}) (Axelsson et al., 1992), but lower than that observed at -1°C by Lowe et al. (2005), also for *P. borchgrevinki* (16 beats min^{-1})

Data on cardiac function in fish that metabolically depress at low temperatures are unfortunately very scarce. The only data I am aware of are for f_H of the goldsinny wrasse (*Ctenolabrus rupestris*), another member of the Labridae family, and the goldfish (*Carassius auratus*). Heart rate in goldsinny fell from 33 to 18 beats min^{-1} between 6 and 4°C, and showed high Q_{10} values, ranging from 5.15 (between 8 and 6°C) to 24.5 (between 6 and 4°C). Moreover, the anoxic goldfish shows a strong correlation between the extent of metabolic depression and f_H (van Ginneken et al., 2004). Cunner cardiac function was also highly correlated with the extent of seasonal changes in routine metabolism (compare Figs. 4.1A and 4.6A and B). For example, Q and f_H both showed a Q_{10} = 2-3 from 14 to 5°C, and when the water temperature fell from 5 to 0°C (fall/winter) both Q and f_H dropped by 50 to 60% (Q > 5), indicative of an active depression of cardiac function. In contrast, stroke volume was insensitive to changes in temperature over the range studied. However, this latter result is not unusual as many fish regulate Q predominantly through adjustments in f_H when faced with acute temperature changes (e.g. Atlantic cod; Gollock et al., 2006) or when acclimated to different temperatures (e.g. largescale sucker, Kolok et al., 1993; largemouth bass, Cooke et al., 2003; winter flounder, Joaquim et al., 2004).

When challenged with an acute temperature decrease, cunner rapidly lowered both Q and f_H, and did not show the initial thermal compensation that was observed for MRr. Further, the Q_{10} value from 5 to 0°C ranged from 2 to 3. This latter result suggesting that when acutely challenged with a temperature drop from 5 to 0°C cunner do not actively depress cardiac function, as was

observed for MRr (Q_{10} ~ 8); i.e. there is a decoupling between MRr and cardiac function. This is in contrast to what was observed with the acute temperature increase, where Q increased rapidly when temperature was raised from 0 to 5°C, and the Q_{10} values for Q and f_H ranged from 5.1 to 6.3 and from 4.4 to 4.7, respectively; this finding being consistent with what was observed with MRr. The only other data on cardiac performance during an acute temperature elevation at these low temperatures come from Antarctic fish, and both studies (Axelsson et al., 1992; Lowe et al., 2005) show that the Q_{10} for changes in cardiac function was 2-3. However, a similar response (i.e. Q_{10} ~2 for f_H and Q) was observed when the winter flounder (*Pseudopleuronectes americanus*) was acutely challenged with an increase in temperature from 5 to 10°C (Cech et al., 1976). These data, although limited, suggest that the cardiac response to an acute temperature increase in cunner is not typical of other teleosts. The reasons for this are not clear, but may be related to alterations in cardiac physiology in the cold or during dormancy.

In fish, as in most species, Q is normally tightly coupled with metabolism (e.g. Webber et al., 1998; Gollock et al., 2006) because an organism's oxygen demand must be met by effective oxygen delivery and the costs of gas exchange, and cardiac pumping, minimized by ventilation-perfusion matching. A tight relationship between Q, f_H and metabolic rate (MRr) was observed in this study when cunner experienced seasonal changes in temperature or when acutely challenged with a temperature increase. In contrast, however, changes in Q and f_H (Q_{10} = 2.1 to 2.9 and 2 to 2.7, respectively) were much less than measured for MRr (Q_{10} ~ 8) when water temperature was acutely decreased from 5 to 0°C. It is unlikely that this mismatch between Q and MRr, when cunner were acutely cooled, is related to the effects of increased blood CO_2 levels or to the effects of stress (i.e. adrenergic stimulation) on cardiac function. First, CO_2 excretion is expected to be rapid

at the low values of Q measured in cunner at 5 to 0°C (see Desforges et al., 2002), further, research on *Squalus acanthias* (Perry and McKendry, 2001) and *Oncorhynchus mykiss* (McKendry and Perry, 2001) indicate that cardiovascular responses associated with hypercarbia are mediated solely by externally oriented receptors. Second, although rapid exposure to cold temperatures induces a stress response in carp (Tanck et al., 2000) the time-frame over which temperature was lowered was much longer in this study (5°C decrease in 5 hours vs. 7 to 11°C in 1 hour), and based on the rapid clearance rates for catecholamines from the blood (Gamperl et al., 1994), it is unlikely that catecholamine levels would be maintained at a concentration that would influence cardiac function for an extended period. Furthermore, the research of Haverinen and Vornanen (2007) indicates that the elevated heart rate (and thus Q) measured in cold-exposed cunner was not the result of insufficient time for the heart's pacemaker cells to remodel their cellular physiology. These authors showed that pacemaker cells from rainbow trout acclimated to cold temperatures actually have higher intrinsic beating rates than those isolated from warm acclimated animals. In some fish, thermal compensation of heart rate following temperature acclimation results from a cold-induced decrease in the inhibitory cholinergic control (Seibert, 1979; Sureau et al., 1989). While a decrease in cholinergic tone is a probable explanation for the higher than expected Q and f_H in cunner acutely exposed to a drop in temperature (from 5 to 0°C), this hypothesis will require experimental validation.

5.3 Research Perspectives

This study together with previous work performed on cunner, suggests that this species is a good model for studying various aspects of metabolic depression in fish, and several research

questions readily come to mind. For example: 1) how are cunner able to achieve this hypometabolic state? (i.e. what aspects of cellular and/or tissue regulation are involved); and 2) how do they know when to employ it ? (i.e. what environmental cues lead to the transition from a normal to an hypometabolic state)

As previously stated, the transition from an active to a hypometabolic state requires a coordinated rebalancing of both ATP consumption and production; i.e. the inhibition of specific cellular processes, as well as a re-arrangement of priorities for energy expenditure is needed to achieve a new rate of ATP-turnover that will sustain a long-term hypometabolic state (Storey and Storey, 2004). Although the proportion of ATP-turnover devoted to specific cellular processes has been examined extensively in mammals and reptiles (Brown, 1992; Rolfe and Brown, 1997; Hulbert and Else, 2004), the energy devoted to some processes may not be the same in fish. For example, although mammals and reptiles devote similar proportions of oxygen consumption (40 - 46%) to processes not coupled to ATP production (e.g. proton leak and non-mitochondrial respiration) (Hulbert and Else, 2004), this value is about 70% in trout myocytes (Mortenssen and Gesser, 1999). Clearly, more studies are needed in fish to identify the cellular processes associated with metabolic depression, and to what extent the energy devoted to these processes is affected when in a hypometabolic state.

Another obvious question is what triggers this hypometabolic state, i.e. how do cunner know when to actively downregulate their metabolic rate. Falling temperatures are a likely trigger, since research in mammals suggests that vertebrates first detect thermal change in peripheral warm and cold receptors located in basal epidermal cells (Crawshaw, 1980). Further, studies in the common carp (*Cyprinos carpio* L.) have shown that the respiratory system is extremely sensitive to changes

in environmental temperature, and that metabolic activity in the brain increases 30s after the onset of a temperature drop (Crawshaw, 1976; van den Burg et al., 2006). Therefore, it is possible that there is a specific temperature below which cunner initiate metabolic depression. However, this would require a high level of plasticity between populations, since the temperature at which whole-body metabolic depression is noticeable differs between geographical distinct populations. On the other hand, photoperiod, in addition to or in conjunction with temperature may initiate winter dormancy in cunner. For example, studies on goldfish (*Carassius auratus*) and tench (*Tinca tinca*) showed that melatonin, a hormone regulated in association with the light:dark cycle, affects food intake and locomotor activity (López-Olmeda et al., 2006). While, the present study showed that an acute temperature decrease was enough to induce cunner to actively depress routine metabolism after a few hours, this experiment was performed in fall fish, and it is possible that photoperiod had already had some influence on the cunner's physiology. Thus, it would be interesting to see if the cunner is able to rapidly depress its metabolism at other times of year (i.e. at different photoperiods).

Finally, although this species does not naturally experience hypoxia, there are recent data to suggest that it has an enhanced hypoxia tolerance. Corkum and Gamperl (unpublished) found that 8°C acclimated cunner could maintain MRr at normoxic levels until a water O_2 saturation of 20%, that the critical O_2 saturation (*Scrit*) at 8 and 1°C was 21.4 ± 0.6 and 16.9 ± 3.1%, respectively, and that between 20 and 10% O_2 saturation they reduced MRr by 42.89 and 23.8%, respectively. This was in contrast with the Greenland cod, which showed *Scrit* values of 54.8 ± 3.4 and 40.4 ± 3.3 % at 8 and 1°C, respectively. Further, preliminary experiments indicate that cunner at 8°C are able to survive for at least for 7 hours after an acute drop in water O_2 saturation to 10% (J. Lewis,

personal communication). The degree to which cunner actively depresses cellular processes under hypoxia is not yet known. However, at least 120 genes are regulated by hypoxia in the euryoxic fish *Gillichthys mirabilis*, including genes associated with the muscle's translation machinery (Gracey et al., 2001), and this tissue appears to be a major target of suppression in hypometabolic states associated with both anoxia and low temperature (Smith et al., 1996; Lewis and Driedzic, submitted). This research suggests that experiments should be conducted to examine whether the regulation of gene expression that leads to a hypometabolic state is similar in animals that undergo metabolic depression as a response to different environmental stressors (i.e. low oxygen and low temperature).

In conclusion, there are a number of interesting questions regarding metabolic depression in fish that could potentially be answered using the cunner, not only due to their unusual response to low temperature (i.e. metabolic depression), but also due to their apparent capacity to tolerate other environmental stressors (i.e. hypoxia) to which they are not naturally exposed.

6. Summary

This study shows that cunner, in Newfoundland, actively depress routine metabolic rate (MRr) and cardiac function when seasonal ocean temperatures fall below 5°C. This conclusion is based on the high Q_{10} (>5) values between 5°C (fall) and 0°C (winter) for MRr, cardiac output (Q) and heart rate (f_H), but not stroke volume (SV_H) which was insensitive to temperature changes. Between 9°C (late spring) and 14°C (summer), and between 14°C and 5°C (fall), cunner showed expected Q_{10} values (i.e. 2-3). However, MRr at 5°C in the spring, when the fish were coming out of dormancy, was significantly lower than MRr in the fall, at the same temperature. This was probably related to the nutritional status of the animals, after 6 months of dormancy, and suggests that these fish are slow 'arousing' from their metabolically depressed state. Further, when temperature increased from 5 to 9°C in the spring, cunner MRr showed a high Q_{10} value (6.3). Based on recent data for protein synthesis in this species we hypothesize that this is related with compensatory growth, after such a long period of inactivity with little or no feeding. The response to the acute temperature challenges was also very interesting, and indicates that cunner have a very plastic regulation of MRr at cold temperatures. When exposed to acute temperature challenges (5 to 0°C or 0 to 5°C) cunner were able to decrease or increase MRr within a few hours or as soon as the final temperature (0 or 5°C) was reached. While cardiac function followed MRr in the acute temperature increase challenge, rapidly increasing as the temperature was elevated, cunner MRr and cardiac function became decoupled when challenged with an acute temperature decrease. The cunner was only able to decrease MRr after 2 hours at 0°C, while both cardiac output and f_H dropped linearly with temperature and remained constant during the 3 hours at 0°C. Further, the Q_{10} values for MRr between 5 and 0°C were ~8, while those for Q and f_H ranged between 2 and 3.

7. Literature Cited

Alkanani, T., Parrish, C. C., Rodnick, K. J. and Gamperl, A. K. 2005. Lipid class and nonesterified fatty acid profiles in plasma of North Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 2509-2518

Axelsson, M. 2005. The Circulatory System and its Control. In: The Physiology of Polar Fishes. A. P. Farrell and J. F. Steffensen (Eds.). Fish Physiology Series Vol. 22. D. J. Randall and A. P. Farrell. Elsevier Academic Press, California, USA. pp. 239-280

Axelsson, M., Davison, W., Forster, M. E. and Farrell, A. P. 1992. Cardiovascular responses of the Red-blooded Antarctic fishes, *Pagothenia bernacchii* and *Pagothenia borchgrevinki*. *The Journal of Experimental Biology* **167**: 179-201

Boutilier, R. G. 2001. Mechanisms of cell survival in hypoxia and hypothermia. *The Journal of Experimental Biology* **203**: 3171-3181

Bridges, C. R. 1988. Respiratory Adaptation in Intertidal Fish. *American Zoologist* **28**(1): 79-96

Brodeur, J. C., Dixon, D. G. and McKinley, R. S. 2001. Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. *Journal of Fish Biology* **58**: 439-452

Brown, G. C. 1992. Control of respiration and ATP synthesis in mammalian mitochondria and cells. *Biochemistry Journal* **284**: 1-13

Bushnell, P. G., Steffensen, J. F., Schurmann, H. and Jones D. R. 1994. Exercise metabolism in two species of cod in artic waters. *Polar Biology* **14**: 43-48

Cech Jr., J. J. 1990. Respirometry. In: Methods for Fish Biology. C. B. Schreck and P. B. Moyle (Eds). American Fisheries Society, Bethesda, Maryland, USA. pp. 335-362

Cech Jr, J. J., Bridges, D. W., Rowell, D. M. and Balzer, P. J. 1976. Cardiovascular responses of winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Canadian Journal of Zoology* **54**: 1383 – 1388

Chiasson, W. B. 1995. Age validation and comparison of growth rates in behaviourally and geographically distinct populations of cunner, *Tautogolabrus adspersus*. M.Sc. Thesis Dissertation, Memorial University of Newfoundland

Clarke, A. and Johnston, N. M. 1999. Scaling of metabolic rate and body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**: 893-905

Cooke, S. J., Ostrand, K. G., Schreer, J. F., Wahl, D. H. and Philipp, D. P. 2003. Cardiovascular Responses of Largemouth Bass to Exhaustive Exercise and Brief Air Exposure over a Range of Water Temperatures. *Transactions of the American Fisheries Society* **132**: 1154-1165

Crawshaw, L. I. 1976. Effect of rapid temperature change on mean body temperature and gill ventilation in carp. *American Journal of Physiology* **231**(3): 837-841

Crawshaw, L. I. 1980. Temperature Regulation in Vertebrates. *Annual Reviews Physiology* **42**: 473-491

Crawshaw, L. I. 1984. Low-temperature dormancy in fish. *The American Physiological Society* R479-R486

Crawshaw, L. I., Lemons, D. E., Parmer, M. and Messing, J. M. 1982. Behavioural and Metabolic Aspects to Low Temperature Dormancy in the Brown Bullhead, *Ictalurus nebulosus*. *Journal of Comparative Physiology* **148**: 41-47

Crockett, E. L. and Londraville, R. L. 2006. Temperature. In: The Physiology of Fishes 3rd Edition. D. H. Evans and J. B. Claiborne (Eds.) CRC Press, Florida, USA. pp.231-269

Curran, M. C. 1992. The Behavioral Physiology of Labroid Fishes. Ph. D. Thesis. MIT/WHOI, WHOI-92-41

Desforges, P. R., Harman, S. S., Gilmour, K. M. and Perry, S. F. 2002. The sensitivity of CO_2 excretion to changes in blood flow in rainbow trout is determined by carbonic anhydrase availability. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **282**: 501-508

Donohoe, P. H. and Boutilier, R. G. 1998. The protective effects of metabolic rate depression in hypoxic cold submerged frogs. *Respiration Physiology* **111**: 325-336

Donohoe, P. H., West, T. G. and Boutilier, R. G. 1998. Respiratory, metabolic, and acid-base correlates of aerobic metabolic rate reduction in overwintering frogs. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **274**: 704 - 710.

Driedzic, W. R. and Gesser, H. 1994. Energy Metabolism and Contractility in Ectothermic Vertebrate Hearts: Hypoxia, Acidosis and Low Temperature. *Physiological Reviews* **74**(1): 221-258

Gamperl, A. K. and Farrell, A. P. 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. *The Journal of Experimental Biology* **207**: 2539-2550

Gamperl, A. K., Vijayan, M. M. and Boutilier, R. G. 1994. Experimental control of stress formone levels in fishes: Techniques and applications. *Reviews in Fish Biology and Fisheries* **4**: 215-255

Geiser, F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Reviews Physiology* **66**: 239-274

Gollock, M. J., Currie, S., Petersen, L. H. and Gamperl, A. K. 2006. Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. *The Journal of Experimental Biology* **209**: 2961-2970

Gracey, A. Y., Troll, J. V. and Somero, G. N. 2001. Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis. Procedings of the National Academy of Sciences* **98**: 1993-1998

Green, J. M. 1975. Restricted movements and homing of the cunner, (Pisces: Labridae). *Canadian Journal of Zoology* **53**: 1427-1431.

Green, J. M. and Farwell, M. 1971. Winter habits of the cunner, *Tautogolabrus adspersus* (Walbaum, 1792), in Newfoundland. *Canadian Journal of Zoology* **49**: 1497-1499

Guderley, H. 2004. Metabolic responses to low temperature in fish muscle. *Biological Review* **79**: 409-427

Guderley, H. and St-Pierre, J. 2002. Review: Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *Journal of Experimental Biology* **205**: 2237-2249

Guppy, M. 2004. The biochemistry of metabolic depression: a history of perceptions. *Comparative Biochemistry and Physiology* B **139**: 435-442

Guppy, M. and Withers, P. 1999. Metabolic depression in animals: Physiological perspectives and biochemical generalizations. *Biological Review* **74**: 1-40

Guppy, M., Fuery, C. J. and Flanigan, J. E. 1994. Biochemical principles of metabolic depression. *Comparative Biochemistry and Physiology* Part B **109**: 175-189

Hand, S. C. and Hardewig, I. 1996. Downregulation of cellular metabolism during environmental stress: Mechanisms and implications. *Annual Reviews Physiology* **58**: 539-563

Haugaard, N. and Irving, L. 1943. Influence of temperature upon the oxygen consumption of the cunner (*Tautogolabrus adspersus*) in summer and winter. *Journal of Cellular and Comparative Physiology* **21**: 19-26

Haverinen, J and Vornanen, M. 2007. Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *American Journal of Physioloy - Regulatory, Integrative and Comparative Physiology* **292**: R1023-R1032

Holeton, G. F. 1974. Metabolic cold adaptation of polar fish: fact or artifact?. *Physiological Zoology* **47**:137-152

Hulbert, A. J. and Else, P. L. 2004. Basal Metabolic Rate: History, Composition, Regulation, and Usefulness. *Physiological and Biochemical Zoology* **77**(6): 869-876

Jackson, D. C. 2000. Living without oxygen: lessons from the freshwater turtle. *Comparative Biochemistry and Physiology* A **125**: 299-315

Joaquim, N., Wagner, G. N. and Gamperl, A. K. 2004. Cardiac function and critical swimming speed of the winter flounder (*Pleuronectes americanus*) at two temperatures. *Comparative Biochemistry and Physiology* A **138 (A)**: 277-285

Johansson, D., Nilsson, G. E. and Tornblom, E. 1995. Effects of anoxia on energy metabolism in crucian carp brain slices studied with microcalorimetry. *The Journal of Experimental Biology* **198**: 853-859.

Killen, S. S., Costa, I., Brown, J. A. and Gamperl., A. K. 2007. *Proceedings of the Royal Society B* **274:** 431-438

Kolok, A. S., Spooner, R. M. and Farrell, A. P. 1993. The Effect of Exercise on the Cardiac Output and Blood Flow Distribution of the Largescale Sucker *Catostomus macrocheilus*. *The Journal of Experimental Biology* **183**: 301-321

Levesque, H. M., Short, C., Moon, T. W., Ballantyne, J.S. and Driedzic, W. R. 2005. Effects of seasonal temperature and photoperiod on Atlantic cod (*Gadus morhua*). I. Morphometric parameters and metabolites. *Canadian Journal of Fisheries and Aquatic Sciences* **62**: 2854-2863

Lewis, J. and Driedzic, W.R. Tissue specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus*. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. In Press*

Lillywhite, H. B., Zippel, K. C. and Farrell, A. P. 1999. Resting and maximal heart rates in ectothermic vertebrates. *Comparative Biochemistry and Physiology* A **124**: 269-382

López-Olmeda, J. F., Madrid, J. A. and Sánchez-Vázquez, F. J. 2006. Melatonin effects on food intake and activity rhythms in two fish species with different activity patterns: Diurnal (goldfish)

and nocturnal (tench). *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **144**(2): 180-187

Lowe, C. J., Seebacher, F. and Davison, W. 2005. Thermal sensitivity of heart rate and insensitivity of blood pressure in the Antartic nototheniid fish *Pagothenia borchgrevinki. Journal of Comparative Physiology* B **175**: 97-105

McKendry, J. E. and Perry, S. F. 2001. Cardiovascular effects of hypercarbia in rainbow trout (*Oncorhynchus mykiss*): A role for externally oriented chemoreceptores. *The Journal of Experimental Biology* **204**: 115-125

Mortenssen, B. and Gesser, H. 1999. O_2 Consumption and Metabolic Activities in Resting Cardiac Myocytes From Rainbow Trout. *Journal of Experimental Zoology* **283**: 501-509

Muusze, B., Marcon, J., van den Thillart, G. and Almeida-Val, Vera. 1998. Hypoxia tolerance of Amazon fish Respirometry and energy metabolism of the cichlid *Astronotus ocellatus. Comparative Biochemistry and Physiology* **120** (A): 151-156

Nilsson, G. E. and Gillian M. C. Renshaw, G. M. C. 2004. Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. *The Journal of Experimental Biology* **207**: 3131-3139.

Olson, K. R. and Farrell, A. P. 2006. The Cardiovascular System. In: The Physiology of Fishes 3rd Edition. D. H. Evans and J. B. Claiborne (Eds.) CRC Press, Florida, USA. pp.119-152

Peck, L. S. 2002. Ecophysiology of Antartic marine ectotherms: limits to life. *Polar Biology* **25**: 31-40

Perry, S. F. and McKendry, J. E. 2001. The relative roles of external and internal CO_2 versus H+ in eliciting the cardiorespiratory responses of *Salmo salar* and *Squalus acanthias* to hypercarbia. *The Journal of Experimental Biology* **204**: 3963-3871

Rome, L. C., Stevens, E. D. and John-Alder, H. B. 1992. The influence of temperature and thermal acclimation on physiological function. In: Environmental Biology of the Amphibians. M. E. Feder and W. W. Burggren (Eds) Chicago, University of Chicago Press. pp 183-205

Rolfe, D. F. S. and Brown, G. C. 1997. Cellular Energy Utilization and Molecular Origin of Standard Metabolic Rate in Mammals. *Physiological Reviews* **77**(3): 731-758

Sayer, M. D. J. and Davenport, J. 1996. Hypometabolism in torpid goldsinny wrasse subject to rapid reductions in seawater temperature. *Journal of Fish Biology* **49**: 64-75

Schurmann, H. and Steffensen, J. F. 1997. Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology* **50**: 1166-1180

Scott, WB and Scott, MG. 1988. Atlantic Fishes of Canada. *Can. Bull.Fish. Aquat. Sci.* **219**: 731p.

Seibert, H. 1979. Thermal adaptation of heart rate and its parasympathetic control in the European eel *Anguilla anguilla*. *Comparative Biochemistry and Physiology* C **64**: 275-278

Smith, H. W. 1930. Metabolism of the lung-fish *Protopterus aethiopicus*. *Journal of Biological Chemistry* **88**: 97-130

Smith, R. W., Houlihan, D. F., Nilsson, G. E. and Brechin, J. G. 1996. Tissue-specific changes in protein synthesis rates in vivo during anoxia in crucian carp. *American Journal of Physiology* **271**: R897-R904

Steffensen, J. F. 2002. Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artifact? Artefact!. *Comparative Biochemistry and Physiology* A **132**: 789-795

Steffensen, J. F. 2005. Respiratory systems and Metabolic rates. In: The Physiology of Polar Fishes. A. P. Farrel and J. F. Steffensen (Eds.). Fish Physiology Series Vol. 22. D. J. Randall and A. P. Farrell, Elsevier Academic Press, California, USA 203-238pp.

Storey, K. B. 2001. Turning down the fires of life: metabolic regulation o hibernation and estivation. In: Molecular Mechanisms of Metabolic Arrest. K. B. Storey (Ed). UK:BIOS Scientific. Oxford. United Kingdom. pp 1-21

Storey, K. B. and Storey, J. M. 1990. Metabolic Rate Depression and Biochemical Adaptation in Anaerobiosis, Hibernation and Estivation. *The Quarterly Review of Biology* **65**(2): 145-174

Storey, K. B. and Storey, J. M. 2004. Metabolic rate depression in animals: transcriptional and translational controls. *Biological Review* **79**: 207-233

Sureau, D., Lagardere, J. P. and Pennec, J. P. 1989. Heart rate and its cholinergic control in the sole (Solea vulgaris), acclimatized to different temperatures. *Comparative Biochemistry and Physiology* A **92**: 49-51

Tanck, N. W. T., Booms, G. H. R., Eding, E. H., Wendelaar Bonga, S. E. and Komen, J. 2000. Cold shocks: a stressor for common carp. *Journal of Fish Biology* **57**: 881-894

Ultsch, GR. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. *Biological Review* **64**: 435-516

van den Burg, E. H., Verhoye, M., Peeters, R. R., Meek, J., Flik, G. and Van der Linden, A. 2006. Activation of a sensorimotor pathway in response to water temperature drop in a teleost fish. *The Journal of Experimental Biology* **209**: 2015-2024

van Ginneken, V. J. T., Snelderwaard, P., van der Linden, R., van der Reijden, N., van der Thillart, G. E. E. J. M. and Kramer, K. 2004. Coupling of heart rate with metabolic depression in fish: a radiotelemetric and calorimetric study. *Thermochimica Acta* **414**: 1-10

Van Waversveld, J., Addink, A. D. F. and Van den Thillart, G. 1989. Simultaneous Direct and Indirect Calorimetry on Normoxic and Anoxic Goldfish. *The Journal of Experimental Biology* **142**: 325-335

Walsh, P. J., Foster, G. D. and Moon, T. W. 1983. The effects of temperature on metabolism of the American eel *Anguilla rostrata* (LeSueur): Compensation in the summer and Torpor in the winter. *Physiological Zoology* **56**(4): 532-540

Webber, D. M., Boutilier, R. G. and Kerr, S. R. 1998. Cardiac output as a predictor of metabolic rate in cod *Gadus morhua*. *The Journal of Experimental Biology* **201**: 2779-2789

Appendix A

Table A.1 – Number of animals (N), mass (g), length (cm) and female to male ratio in each of the experimental groups; ¥ indicates number of fish where sex determination was not possible; (*) 3 cunner, from the initial group of 10, died during the experiment between measurements. One-way ANOVA was used to test for differences between mass and length within the Routine Metabolic Rate or Cardiac Function groups. Values are means ± (SE); different letters indicate significant differences ($P < 0.05$) between groups within the routine metabolic rate and cardiac function experiments.

GROUPS		N	Mass (g)	Length (cm)	Female/Male
Routine Metabolic Rate	**Seasonal**	7*	224.4 (16.5)^{a}	24 (0.5)^{a}	4/3
	Acute decrease	8	190.4 (18.4)^{a}	24 (0.7)^{a}	4/3 (1)$^{¥}$
	Acute increase	8	137.9 (12.5)^{b}	21 (0.5)^{b}	5/2 (1)$^{¥}$
Cardiac Function	**14°C**	7	270.9 (13.9)^{a}	27 (0.3)^{a}	¾
	5°C	7	219.9 (20.5)^{a}	23 (0.9)^{b}	5/2
	0°C	7	227.4 (6.0)^{b}	25 (0.6)^{b}	4/3

Table A.2 – Mass (g) of the fish used in the assessment of seasonal change in routine metabolic rate (n=7). A repeated measures one-way ANOVA, followed by a Tukey post-hoc test, was used to test for differences between mass at different temperatures/seasons. Values are means (± SE); different letters indicate significant differences ($P < 0.05$).

	Temperature (°C) / Season				
	5 (spring)	**9** (spring/summer)	**14** (summer)	**5** (fall)	**0** (winter)
Mass (g)	240.1 (20.2)^{a}	232.9 (18.2)^{ab}	217.1 (17.6)^{b}	220.6 (15.8)^{ab}	210.6 (14.9)^{b}

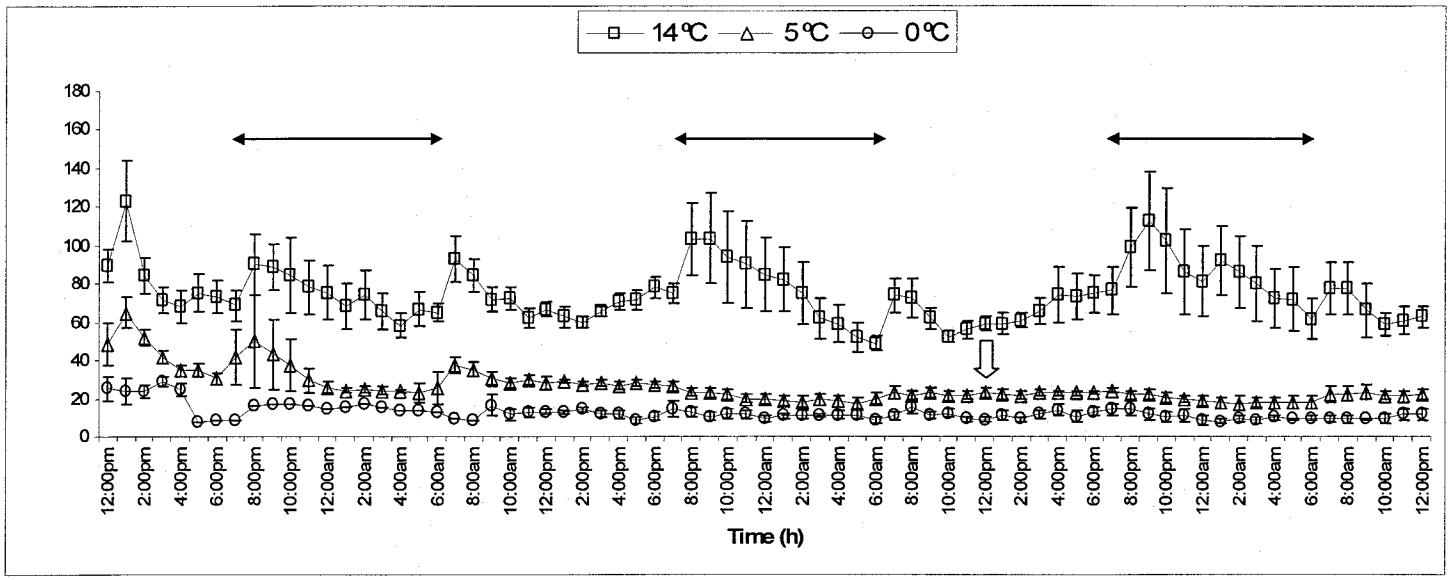


Figure B.1 – Routine Metabolic Rate of cunner at 5°C (during the spring, △), at 14°C (during late summer, □) and at 0°C (in the winter, ○). The horizontal black arrows indicate measurements made during the night for the 14°C fish. The vertical arrow indicates 48 hours, the period determined in the first measurement period (spring, 5°C) to be required for the fish to fully habituate to the condition within the respirometer. Note: the increased variability in MO_2, corresponding to time of day (at 14°C), was not statistically significant).

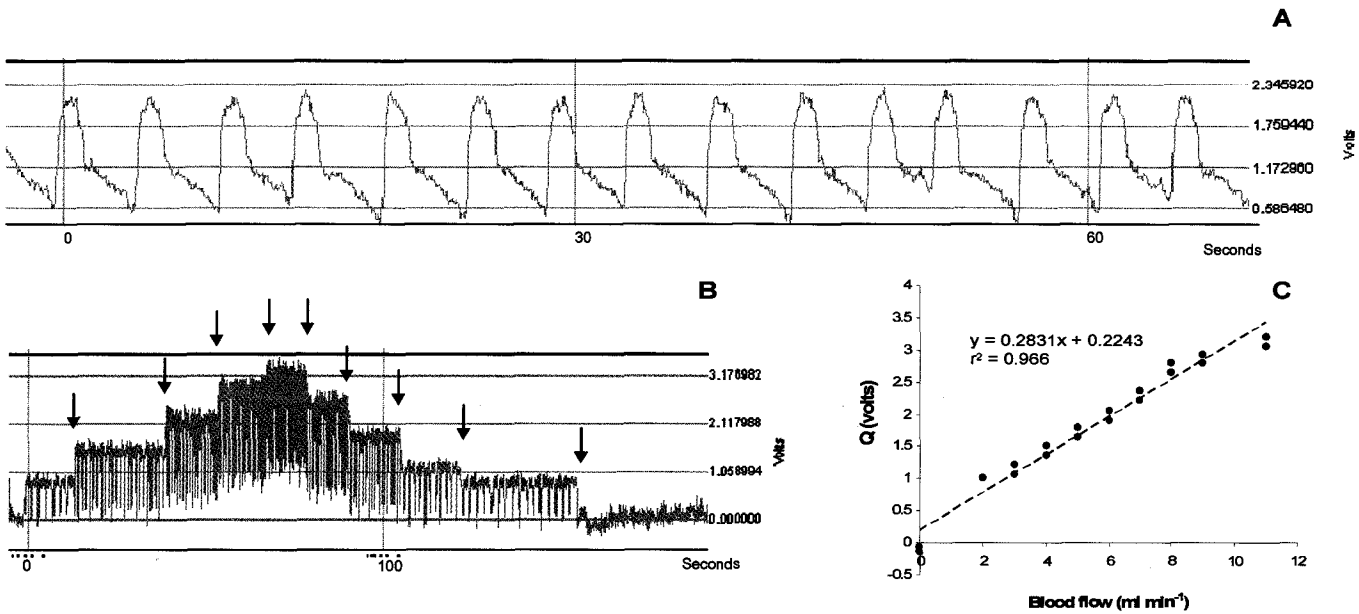


Figure C.1 – Determination of absolute cardiac output (Q, ml min^{-1}). The Doppler flow probe emits ultrasound waves, from a vibrating crystal, that are reflected by passing blood cells, resulting in a signal frequency shifts. These shifts in frequency represent velocity and are measured as a change in voltage. Peaks in voltage indicate a heart beat, while the mean voltage per unit of time is an indication of cardiac output (Cooke et al., 2003). A) Sample of the raw signal obtained from a cunner, implanted with a Doppler flow probe, showing the fish's heart rate (systolic peaks) and cardiac output (in volts). During calibration of the flow probe (B), blood is artificially pumped through the cannulated heart, with a peristaltic pump, with the probe in place, at several known flow rates; the black arrows in B indicate the establishment of a new flow rate. Finally, the linear regression between mean voltage output and blood flow (C) results in an equation that is then used to convert the raw signal (Q, volts), for individual fish, into absolute blood flow or cardiac output (Q, ml min^{-1}).