

THE BEHAVIORAL PHYSIOLOGY OF LABROID FISHES

by

Mary Carla Curran

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Certified by

*PSL*

Phillip S. Lobel  
Thesis Advisor

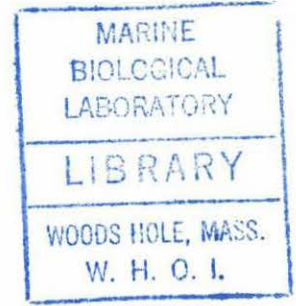
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Chairman, Joint Committee for Biological Oceanography,  
Massachusetts Institute of Technology/  
Woods Hole Oceanographic Institution



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Mary Carla Curran

Submitted to the Massachusetts Institute of Technology/  
Woods Hole Oceanographic Institution  
Joint Program in Biological Oceanography  
in partial fulfillment of the requirements for  
the degree of Doctor of Philosophy

## ABSTRACT

The family Labridae, or wrasses, is one of the most speciose fish families and is exceptional in its wide range of morphological and behavioral diversities. The cunner *Tautoglabrus adspersus* is one of two temperate-dwelling Western North Atlantic representatives of this family, and they are one of the few fishes that remain in New England waters throughout the year. In the winter, the cunner enters a state of "torpor" which has previously been described based solely on behavioral observations. The present study showed that cunner undergo physiological torpor, or hibernation, based on low oxygen consumption rates in winter, contributing to a large  $Q_{10}$  value of 8.5. It is thus established as one of the few marine species that is known to hibernate.

Cunner withstood four months of starvation at 4°C. Glycogen, lipid, and protein in the liver decreased during this period, as did the liver/body ratio, but these components did not decrease significantly in the whole-body samples. Since liver components were not exhausted, and body components were not significantly affected, cunner can withstand long periods without eating. Regression analysis predicts that they can live at least 6 months given the rate of decrease of glycogen and lipid reserves, and 9 months based on their protein reserves.

Oxygen consumption rates were monitored continuously over several days to determine diel variations in metabolic rate. The values obtained at night were significantly lower than the daytime values. Cunner did not maintain a diel cycle throughout the year; the length of this cycle varied from approximately 24 hours during warm temperatures to approximately 48 hours at temperatures generally below 8°C. Metabolic rates were more variable at warmer temperatures, which is in agreement with the expected increase in spontaneous activity.

Two tropical labroids, the wrasse *Thalassoma bifasciatum* and the parrotfish *Scarus iserti*, also had significantly higher oxygen consumption rates during the day than at night. Both hibernation and sleep are thought to be energy conserving mechanisms in fishes. The ability of labrids to sleep may have predisposed them to becoming established in temperate waters by surviving cold temperatures through hibernation.

Thesis Supervisor: Phillip S. Lobel ·  
Title: Associate Scientist  
Woods Hole Oceanographic Institution

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## CHAPTER 1

### INTRODUCTION

My dissertation incorporates several topics in the fields of behavior and physiology that relate to cyclical changes in activity and metabolism of fishes in tropical and temperate waters. The study focuses on two families of fishes (labrids and scarids) that are evolutionarily of tropical origin, although there are two species found in the Western North Atlantic. A striking characteristic of these species is their marked inactivity at night. In fact, they exhibit several characteristics of mammalian sleep. The ability of some labrids and scarids to bury themselves in the sediment, or secrete a mucous cocoon at night or when oxygen tension is low, indicates that these fishes may be able to reduce their metabolic rates more than other species. The presence of some underlying mechanism enabling a metabolic reduction is also exemplified in temperate labrids such as the cunner *Tautoglabrus adspersus*; they undergo torpor in the winter. Until this study, it was unknown if cunner hibernate (i.e., metabolic rates being reduced more than expected based on a decrease in temperature). The ability of labroid fishes to undergo short periods of metabolic reduction (sleep) may have predisposed members of this group toward hibernation, thereby providing substantial energy savings. Although this evolutionary hypothesis is not testable, it was possible to determine if cunner hibernate, as well as to determine the diel changes in metabolic rates of tropical and temperate fishes. The implications of this research are far-reaching. Potential limitations on the biogeographical distribution of cunner posed by hibernation and the concomitant period

of starvation were established. The ability of marine fishes to hibernate has not been adequately addressed. Hibernation is one of the main methods that animals employ during cold temperatures, apart from maintaining metabolic functions (at an extreme cost particularly when food is limiting) or migration. In order to understand how animals hibernate it is essential to know baseline metabolic rates so that minimal requirements for survival can be established.

The focus of this study was to investigate diel differences in the physiology of nocturnally-resting labrids and scarids (Chapters 4 and 5), and seasonal differences in cunner (Chapter 3) in order to determine the degree to which metabolism is reduced. Changes in metabolism were measured by obtaining oxygen consumption rates for temperate and tropical species, and  $Q_{10}$  values for temperate species. A strong case supporting hibernation in this group would be if  $Q_{10}$  values were significantly greater than 2 or 3. A  $Q_{10}$  of 8.5 for cunner was calculated between 6.4-12°C indicating that oxygen consumption is strongly affected by temperature and that at temperatures below 6.4°C, cunner hibernate. This is in keeping with observations by previous investigators who noted the animals were behaviorally torpid.

Because of the cessation of feeding during hibernation, the effect of starvation on cunner during winter was also determined (Chapter 4). As food deprivation may last for several months, the ability of an individual to survive will greatly depend on stored food reserves. The three main energy reserves in fishes are glycogen, lipid, and protein. The liver concentration of all three components decreased in four months, whereas there was little change in the muscle components. By calculating the rate of decrease for these

reserves, cunner could live 6, 6, and 9 months respectively on their glycogen, lipid, and protein stores in the liver. This finding implies that although some stored energy is depleted, cunner are capable of surviving several months at 4°C without eating and therefore their winter survival would not be limited by energy storage. These results also indicate that the biogeographic range of cunner would not be restricted because of extended periods of cold water temperatures; cunner are capable of surviving many months at low temperatures.

The amount of energy expended during wakefulness is affected by activity level. Sleep and rest are ways to reduce energy expenditure without undergoing long periods of inactivity, such as hibernation. Both the cunner (Chapter 5) and tropical labroid fishes (Chapter 6) experienced diurnal metabolic cycles with lower metabolic rates at night. As labrid fishes are particularly inactive at night, and some species bury themselves in the sand or construct mucous cocoons, the potential effect this ability could have on hibernation in labroids intrigued me and was the impetus for this study.





## CHAPTER 2

### BACKGROUND

#### SUBORDER LABROIDEI

Fishes in the suborder Labroidei demonstrate cyclical changes in metabolism that raise questions about the physiological and behavioral characteristics of these cycles. This suborder is comprised of the families Labridae, Scaridae, Odacidae, Embiotocidae, Pomacentridae and Cichlidae. It contains a substantial portion (5-10%) of all living fishes, approximately 1470 species, in part because it contains two of the most speciose families of fishes: cichlids and labrids (Kaufman and Liem, 1982). Two families are addressed in this dissertation: the Labridae and Scaridae. The family Labridae is the second largest family of marine fishes, containing 500-600 species (Gosline and Brock, 1960; Nelson, 1984). The family Scaridae has approximately 80 species (Gosline and Brock, 1960; Bohlke and Chaplin, 1968). Some systematists place the Scaridae as well as the Odacidae into the family Labridae, based on morphological and functional characters of bones and muscles (Kaufman and Liem, 1982). Specifically, toothplates of the fourth pharyngobranchials are absent in all these fishes, and there is physical contact between the lower pharyngeal jaw and cleithrum. The separation of labrids and scarids into two distinct families is based on feeding habits rather than morphological differences; wrasses are carnivores and parrotfishes are herbivores. Hence, this is one of the few cases in which species were placed in different taxa based on feeding habits, not

morphological characters. To avoid confusion in this dissertation, "labrids" will refer only to wrasses.

### **Key Innovations of Labroid Fishes**

A prime example of the importance of a key innovation, a character that ultimately leads to advantages not initially important in the establishment of the character thus enabling the exploitation of another habitat (Boch, 1965), is found in labroid fishes. The feeding apparatus of cichlid fishes enabled them to eat novel prey but also led to their successful occupation of many African lake systems (biogeographic expansion) each with their own unique species composition (Liem, 1974; Liem and Osse, 1975). This feeding mechanism, the pharyngeal jaw apparatus, enabled labroids to eat prey that would otherwise be too difficult to swallow or digest. These jaws are located in the throat and are used to masticate prey. The advent of this muscular system in the Labroidei may have maximized potential feeding strategies of these fishes. Non-labroids have pharyngeal jaws; however since they lack the appropriate muscle attachments, these jaws cannot chew prey. Cocooning behavior and the associated decrease in metabolism may also be an example of a key innovation in labroids, possibly by enabling labrids to become established in North Atlantic waters because of their ability to undergo long periods of metabolic reduction (i.e., hibernation).

### **Behavioral/Physiological Characteristics of Tropical Labrids and Scarids**

Tropical labrids (wrasses) and the closely related family Scaridae (parrotfishes) are diurnal and exhibit three distinct behavioral/physiological traits that are unique to this

group making them ideal subjects for study of diel changes in behavior and physiology.

Some species:

- 1) have settlement-stage larvae that bury themselves in the sand and emerge as juveniles several days later (Victor, 1983);
- 2) are specialized for seeking refuge under sand. These species typically bury themselves each night to sleep, and can dive into the sediment at any time when threatened (Hobson, 1968; 1974); or
- 3) secrete a mucous cocoon at night (Byrne, 1970).

In coral reef communities, labrids are one of the first groups to take cover at night. The smallest species generally take cover first, and within a species, the larger individuals are often the last to retire and the earliest to emerge in the day (Hobson, 1972). Some labrids and scarids rest within crevices in the reef, and there is some degree of site-fidelity (Winn and Bardach, 1960; Hobson, 1972).

Several labrids bury themselves at night or when disturbed (Table 2.1) some leaving their mouths near or at the surface (Longley and Hildebrand, 1941; Breder, 1951; Gosline and Brock, 1960; Bohlke and Chaplin, 1968). All the Hawaiian labrids bury themselves, with the exception of one species that secretes a cocoon (Gosline and Brock, 1960). A

Caribbean species, *Halichoeres bivittatus*, also buries itself and will do so for up to week when blinded (Breder, 1951). An individual transported to Woods Hole from Jamaica remained buried for several days before emerging (pers. obs.).

Only some species of wrasses and parrotfishes are capable of secreting a mucous cocoon (Table 2.2). The cocoon is a thin, clear mucous bubble that encases the fish, except for a flap-covered whole over the mouth, and a small opening at the rear to rid respiratory water (Winn, 1955). The mucus is produced in the opercular cavity by specialized cells arranged in branching tissue or in a compact gland (Byrne, 1970; Casimir, 1971). The mucus is secreted from the mouth and progresses back around the fish (Winn, 1955). Individuals begin secreting the cocoon only if supported on one side of its body (Byrne, 1970). In the laboratory, they may rest against the aquarium wall or seaweed; the pectoral fins are used to direct the cocoon backwards (pers. obs.; B. Heskiss, pers. comm.). The time required to form a cocoon for *Scarus dubius* and *S. perspicillatus* was 69 minutes and 71 minutes, respectively (Byrne, 1970).

The cocoon is usually secreted at night, particularly when the fish has been stressed. Light inhibits cocoon formation and darkness promotes it (Byrne, 1970), although blinded *Scarus vetula* cocooned during the day (Winn and Bardach, 1960). Several scarid species secrete cocoons during the day under anoxic conditions (Winn, 1955; pers. obs.), indicating that its construction may be induced by stress and may ultimately provide metabolic savings.

One hypothesis for the selective advantage of cocoon production is that a cocoon limits diffusion of a fish's scent. The spotted moray eel *Gymnothorax moringa*, a nocturnal predator of labrids and scarids, was less likely to eat a scarid in a cocoon than a scarid species that does not cocoon (Winn and Bardach, 1959). Smaller species members of species are more likely to form cocoons (Hobson and Chave, 1972), possibly to increase protection from predators (Hobson, 1965). Individuals are capable of secreting more than one cocoon at night if they are disturbed and break out of the cocoon (pers. obs.). Some fishes combine cocoon secretion with other protective behaviors. For example, *Scarus taeniurus* secretes a cocoon while in coral crevices (Hobson, 1972), and *Cryptotomus roseus* buries itself before making its cocoon (Bohlke and Chaplin, 1968).

#### **Behavioral/Physiological Characteristics of Temperate Labrids**

The cunner *Tautoglabrus adspersus* and the tautog *Tautoga onitis* are two of the most common species of local inshore fishes, and are often found near rocky outcrops as well as man-made structures such as pilings and artificial reefs (Stone and Clark, 1970; Olla et al., 1974; Serchuk and Cole, 1974; Olla and Samet, 1977). Their presence in temperate waters may be attributable to a key innovation, their ability to undergo hibernation. The cunner is distributed from Newfoundland to the Chesapeake Bay, and tautog range from Nova Scotia to South Carolina (Bigelow and Schroeder, 1953). Tautog can survive in water as cold as 1.9°C (Olla et al., 1980) and in water as warm as 26.8-30°C (Pearse, 1969; Olla and Studholm, 1975). Green and Farwell (1971) observed no cunner mortality in the field nor in the laboratory at temperatures below 0°C.

Cunner and tautog enter a state of "torpor" during the winter months (Green and Farwell, 1971; Chao, 1972; Olla et al., 1980). Although these sources claim that the cunner and tautog enter torpor, they provide no evidence that a physiologically torpid condition exists rather than merely a change in metabolic rate due to a decrease in temperature ( $Q_{10}$  principle). In winter, the younger tautog remain inshore and become lethargic (or enter "torpor") below 5°C (Olla et al., 1974; 1980). These fish may bury themselves in several millimeters of sediment (Olla et al., 1978; Olla et al., 1980), or lie on their sides on the sediment surface (Cooper, 1966; pers. obs.). While SCUBA diving, I was able to hold an unresponsive tautog in my hand for over a minute before it righted itself and was able to swim. Larger tautog are thought to migrate offshore as the water temperature drops below 11°C, and may not enter torpor. They are usually not seen inshore between November and April (Cooper, 1966). In winter, cunner bury themselves in the mud (Hauggaard and Irving, 1948; Olla et al., 1978), situate themselves under loose rocks (Green and Farwell, 1971), or enter rock crevices (pers. obs.). After removing a layer of rocks, several cunner were seen pressed against each other between a group of other rocks. Several individuals were wedged between other rocks. Cunner remain in torpor until the water temperature rises above 5°C in the spring (Green and Farwell, 1971).

## METABOLISM

In order to determine the degree to which metabolic rate decreased in cunner, oxygen consumption rates were obtained over a range of temperatures. Standard Metabolic Rate (SMR) is often the way in which the metabolic rate of ectotherms is reported, and is the lowest metabolic rate under a specified set of conditions (Hochachka and Guppy, 1987). Erroneously high values are often reported because the experimental animal is not at rest. For example, values reported for SMR were 30-40% lower as reported by Ott et al. (1980) than in Winberg (1956) for the trout *Salmo gairdneri*. Some measurements of oxygen consumption of fishes are listed in Table 2.3.

Routine metabolic rate is used to describe the average oxygen consumption rate associated with spontaneous activity (Fry, 1957; Brett, 1972), although others coined this expression to describe the oxygen consumption rate during a broad range of activity levels (Brett, 1972). Routine activity was 43% higher than resting metabolic rate in the snapper *Lutjanus campechanus* (Wakeman et al., 1979). Active metabolic rate is the maximal sustained rate of metabolism (Brett, 1972) and is obviously affected by activity level. It can differ five-fold among species (Brett, 1972). Maximum oxygen consumption rates can be up to 1000 mg O<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup> (Brett, 1972).

Many other factors besides activity level affect the metabolic rate in fishes, including size and temperature. Metabolic rate usually decreases with decreasing temperature and increasing size (on a weight-specific basis)(Huntsman and Sparks, 1924; Saunders, 1963; Prosser, 1986). This weight-specific relationship is represented by the following formula:

$$\text{metabolic rate (mg O}_2\text{)} = aM^b$$

where  $a$  and  $b$  are constants and  $M$  is body weight (g). For fishes, the exponent  $b$  is approximately 0.8 (Winberg, 1956; Saunders, 1963; Muir and Niimi, 1972). On a log-log plot of SMR vs body mass  $b$  is approximately -0.25 (Kleiber, 1961; Brett and Groves, 1979). The constant  $a$ , although more variable, is usually close to 0.3 (Winberg, 1956). The above relationship is cited extensively in the literature and although the values for  $a$  and  $b$  change among species, the general relationship remains (Smith, 1978; Smith and Laver, 1981; Smith and Brown, 1983).

Temperature is another factor that plays a large role in determining the metabolic rate of fishes (Brett, 1972). Usually, metabolic rate increases with increasing temperature. This has been demonstrated in trout *Salmo gairdneri*, carp *Cyprinus carpio*, goldfish *Carassius auratus*, and cod *Gadus morhua* (Saunders, 1963; Ott et al. 1980; Refinetti and Refinetti, 1988).

The effect of temperature on metabolic rate is often represented using the  $Q_{10}$  principle. It states that for every 10°C increase in temperature, there is a 2-3 x increase in metabolism. This effect has been documented in several species including carp, trout and the snapper *Lutjanus campechanus* (Wakeman et al., 1979; Ott et al., 1980). Physiological data such as oxygen consumption rate and heart rate can be used to generate  $Q_{10}$  values. The measurement of oxygen consumption rates is a common and accurate method for determining the metabolic rate of an organism and was the method



used to determine the effect of temperature on the metabolic rate of cunner. A large  $Q_{10}$  value at low temperatures would indicate that cunner hibernate.

Although temperature may play a significant role in affecting metabolic rate, body composition and species habits may also have a large influence on metabolism. In deeper living species, it was thought that respiratory rate decreased with increasing minimum depth of occurrence due to the effect of decreasing temperature, but only 2% of this decrease was attributable to temperature. Thirty percent of the decrease was accounted for by the increased water content of the animal (Torres and Somero, 1988). Neither size nor pressure influenced respiration rates significantly. The lower respiration rates of deep-sea fishes may occur because of lower activity levels, and is advantageous because a larger proportion of available energy may be used for growth and reproduction (Torres and Somero, 1988).

Other factors such as fish handling and feeding also affect respiration rates. Handling can increase  $O_2$  consumption by 70% and have noticeable effects for 3-5 h afterwards (Saunders, 1963). Other authors have also noted that handling affects the fish for 20 min to hours after capture (Smith, 1978; Smith and Laver, 1981; Smith and Brown, 1983; Du Preez et al., 1986). Oxygen consumption of fishes rises after feeding. The effect of food on respiration rates has been documented by Edwards et al. (1969; 1972), who found that the respiration rate was proportional to food intake. Saunders (1963) noted that cod had a peak in oxygen consumption rates 12-24 hours after feeding. Muir and Niimi (1972) also noted an increased oxygen consumption in *Kuhlia sandvicensis* lasting 42-60 hours

after feeding and reaching a maximum about 10-12 hours after being fed. Therefore, fed fishes can have artificially high standard metabolic rates. Du Preez et al. (1986) noted that SMR in fed sparids were 44% higher than those starved, and Sullivan and Smith (1982) noted that starved fish had a low oxygen consumption rate.

The goal of this dissertation was to incorporate the fields of behavior and physiology by determining cyclical changes in activity and metabolism of both tropical and temperate fishes. A study of labroid species provides a unique opportunity to address changes in activity patterns on a diel as well as a seasonal scale. The initial interest in cocooning behavior of tropical fishes, and the associated decrease in metabolism, sparked the investigation into the potential ramifications this behavioral/physiological characteristic had into the establishment of labroids in the Western North Atlantic.

Table 2.1

## Labrid Fishes That Bury Themselves

Species	Source
<i>Oxyjulis californica</i>	Herald, 1961
<i>Thalassoma</i> sp.	Breder, 1951
<i>Halichoeres bivattatus</i>	Breder, 1951
	Tauber and Weitzman, 1969
<i>Hemipteronotus novacula</i>	Bohlke and Chaplin, 1968
<i>Hemipteronotus splendens</i>	Longley and Hildebrand, 1941
	Bohlke and Chaplin, 1968
	Randall, 1968
<i>Hemipteronotus martinicensis</i>	Bohlke and Chaplin, 1968
<i>Cryptotomus roseus</i>	Bohlke and Chaplin, 1968

Table 2.1

A list of the labrid species that bury themselves is reported.

Table 2.2

## Labrids and Scarids Known to Cocoon

Species	Source
<b>Labridae</b>	
<i>Halichoeres garnoti</i>	M. C. Curran, pers. obs.
<i>Labroides pthiophagus</i>	Gosline and Brock, 1960 Byrne 1970 Tinker, 1978
<i>Bodianus diplotaenia</i>	Hobson, 1965
<i>Pimelometopon pulchrum</i>	Hobson, 1965
<i>Thalassoma bifasciatum</i>	Pers. obs.
<b>Scaridae</b>	
<i>Cryptotomus roseus</i>	Bohlke and Chaplin, 1968
<i>Scarus vetula</i>	Winn and Bardach, 1960 Bohlke and Chaplin, 1968 Smith and Tyler, 1972 Burgess et al., 1988
<i>Scarus iserti</i> (= <i>croicensis</i> )	Winn, 1955 Winn and Bardach, 1959 Winn and Bardach, 1960 M. C. Curran, pers. obs. B. Heskiss, pers. comm.
<i>Scarus taeniopterus</i>	Herald, 1961 B. Heskiss, pers. comm.
<i>Scarus guacamaia</i>	Winn, 1955 Herald, 1961 Bohlke and Chaplin, 1968
<i>Scarus californiensis</i>	Hobson, 1965
<i>Scarus taeniurus</i>	Hobson, 1972 Hobson and Chave, 1972
<i>Scarus gibbus</i>	Burgess et al., 1988
<i>Scarus dubius</i>	Byrne, 1970
<i>Scarus perspicillatus</i>	Byrne, 1970
<i>Scarus coeruleus</i>	Bohlke and Chaplin, 1968
<i>Sparisoma rubripinne</i>	Winn, 1955 Winn and Bardach, 1960
<i>Sparisoma aurofrenatum</i>	B. Heskiss, pers. comm.

Table 2.2

The labrids and scarids that have been documented to make a cocoon are reported.

Table 2.3  
Oxygen Consumption Rates of Fishes

SPECIES	TEMP (°C)	WEIGHT (g)	OXYGEN CONSUMPTION (mg O <sub>2</sub> ·kg <sup>-1</sup> ·h <sup>-1</sup> )	SOURCE
<i>Cyprinus carpio</i>	5	1525	14.9	Ott et al., 1980
"	10	1341	30.7	"
"	15	1649	53.4	"
"	25	1553	83.9	"
"	32	1015	131.1	"
<i>Carassius auratus</i>	20	2	257.4	Refinetti and Refinetti, 1988
<i>Salmo gardneri</i>	10	601	41.6	Ott et al., 1980
"	15	561	60.2	"
"	20	542	542	"
<i>Lutjanus campechanus</i>	20	range 235-7005	62	Wakeman et al., 1979
<i>Sarotherodon niloticus</i>	25	77	93	Verheyen et al., 1985

Table 2.3

Oxygen consumption rates for a number of species over a range of temperatures are presented. Some values were converted into mg O<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup> for comparison. In general, oxygen consumption rate increases with increasing temperature.



## CHAPTER 3

### SEASONAL CHANGES IN OXYGEN CONSUMPTION RATES OF THE CUNNER *TAUTOGOLABRUS ADSPERSUS* (WALBAUM) 1792

#### INTRODUCTION

Most of the detailed studies on physiological changes associated with torpor and hibernation has been performed on mammals. "Hibernation" broadly means to pass the winter in sequestration. It is defined in mammals as an inactive period (days or weeks) during which the body temperature ( $T_b$ ) is approximately 5°C. "Torpor" in mammals is also an inactive period during which there is a controlled drop in body temperature, but the drop is not as large nor does the bout last as long (Lyman et al., 1982). There is a fundamental difference between hibernation in mammals and ectotherms because of the need of the former to control body temperature. Because the definitions of hibernation and torpor are frequently based on controlled changes in body temperature, they do not apply as such to ectotherms, which maintain body temperature similar to their external environment. However, the terms do apply when considering the concomitant decrease in metabolism associated with these periods. "Torpor" is described in several fish families, but authors rarely mention what criteria they used to determine if a fish was torpid. Perhaps behavioral activity and metabolism are reduced only as a result of a decrease in temperature ( $Q_{10}$  principle). "Physiological torpor" or "hibernation" can be considered to be indicated by a metabolic decrease greater than that expected over a given

temperature range on the basis of a  $Q_{10}$  affect alone (Ultsch, 1989). Oxygen consumption rates were determined, and  $Q_{10}$  values were calculated to determine whether or not cunner hibernate according to this criterion.

Hibernating ectotherms often encounter temperatures that are low enough to prohibit functions such as movement, reproduction, feeding, and digestion and may remain inactive for 7-8 months (Hochachka and Guppy, 1987). Many terms have been coined to describe this state. Some authors use the term "overwintering" to suggest a behavioral or physiological change in winter, but it simply means what an animal does to pass the winter. Nikolsky (1963) identified overwintering fishes by their reduced activity, little or no food consumption, and a fall in metabolism. Crawshaw (1984) preferred the word "dormancy," meaning "behavioral inactivity," because it has no connotations of changes in body temperature regulation, unlike the words "torpor" or "hibernation," and believes that the entire physiology of the animal is altered. Nikolsky (1963), Crawshaw (1984), and Brett (1972) claim that this dormancy is an adaptation in fishes, induced by changes in temperature and daylength, which ensures survival through periods of low oxygen availability, lack of food, or extreme temperatures. Ultsch (1989) also suggested that modifications in behavior in winter, such as burying in mud, may be associated with changes in temperature, food abundance, oxygen levels, and plant cover. Fishes may reduce activity levels to survive during food shortages (Ultsch, 1989).

Several fish species undergo behavioral or physiological modifications in cold temperatures. Nikolsky (1963) noted overwintering in flatfishes, sturgeons, and carp but



gave no literature citations or any evidence supporting the existence of reduced metabolic activity. Osipova (1979) also noted that the carp overwinters until water temperatures begin to warm in the spring, but again no physiological data were presented. The winter flounder *Pseudopleuronectes americanus* bury themselves and are found 12-15 cm deep in the sediment, presumably to avoid ice contact; the sediment is up to 0.4°C warmer than water temperature (Fletcher, 1977). Eels such as the American eel *Anguilla anguilla* burrow in the mud during winter (Nyman, 1972). Crawshaw (1984) noted dormancy in the brown bullhead *Ictalurus nebulosus* and the largemouth bass *Micropterus salmoides*. Based on  $Q_{10}$  values, some fishes hibernate. In the largemouth bass, food consumption was nearly zero at temperatures below 7°C, the fish entered torpor below 5°C, and they had  $Q_{10}$  values ranging from 7-19 between the lower temperature ranges of 3-5°C, and 7-9°C (Lemons and Crawshaw, 1981). At temperatures below 5°C, the American eel *Anguilla rostrata* ceased eating and buried themselves in the mud. It had a significantly reduced metabolism:  $Q_{10}$  of 4.1 between 5-10°C (Walsh et al., 1983). Food deprivation may have a large role in effecting metabolic reductions in cold temperatures and this is the focus of the research described in Chapter 4.

## MATERIALS AND METHODS

Fish were collected using traps, and maintained and fed in the laboratory for several weeks under ambient temperature and photoperiod before the experiment. Immediately prior to an experiment, fish were weighed, and total and standard length measurements

were made. Fish were then placed in a flow-through respirometry chamber and monitored for several consecutive days, usually 10 to 14 without being fed. The ENDECO computerized oxygen electrode system collected data on the oxygen concentration of the water every fifteen minutes and this information was automatically stored on disk for later analysis. Four oxygen electrodes were used, therefore three fish and one control could be run at any one time. The fish were visually isolated from one another and none of the water passed from one chamber into another. For a control, a chamber identical to the others was used, except no fish was included. This was designed to take into account any respiration from organisms other than the fish. Previous research by Sullivan and Smith (1982) noted that bacteria did not significantly change the oxygen tension of water in a closed chamber over a 48-h period. The water was passed through a 500 um filter, and was supplied to the chambers gravimetrically. The flow rate passing through the chamber was noted every few hours. The rate remained fairly constant and generally varied less than a few tenths of a liter per hour. Problems with blockage in the system were obvious and the data from these time periods were not used. The flow rate was adjusted depending on season (e.g. in cold temperatures a lower flow rate was used) and was needed to calculate oxygen consumption rates. The value for the oxygen concentration of the water in a chamber containing a fish was subtracted from the value of the oxygen concentration in the water of a chamber without a fish to determine the amount of oxygen extracted by the fish. By knowing the flow rate and the amount of oxygen taken from the water, oxygen consumption rates were determined. Mean hourly

rates were obtained using the four-15 min values obtained each hour. The oxygen probes experienced some drift every day and this factor was taken into account by subtracting/adding the drift from the recorded value. This was determined by assuming that the drift was linear and then subtracting/adding the appropriate amount to the consecutive values in the time series. Figure 3.1 is an example of a probe drift that decreases over time. Because small changes in barometric pressure alter oxygen partial pressures only slightly, no correction was made. This is in keeping with methodology cited in the literature (Ultsch and Duke, 1990). All measurements were made at ambient water temperature and photoperiod to best reflect natural conditions.

Once these preliminary results were obtained, the data were converted to a standard measure of oxygen consumption rate ( $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) by using multiple regression analysis. The multiple regression model has been used successfully in the past (Wohlschlag et al., 1968; Cameron, 1969) and is the most accurate method because it controls for the effects of both temperature and weight. Mean oxygen consumption rates for each fish were obtained in order to fit this model. The data on the first day of the experiment were not used because of the effect of handling on metabolism (Saunders, 1963).

## RESULTS

There was a strong relationship between temperature and oxygen consumption rates, although the effect of temperature was not constant throughout the temperature range.

In other words, a better fit of the data was obtained using a two-line model. Only the data below 12°C were used to generate this model for several reasons. There appeared to be a levelling off of the data above 12°C, and this could affect the model over the temperature range of interest (i.e., lower temperatures). Also, more experiments were conducted at the lower temperatures, therefore, temperatures above 12°C were not as well represented. Because it appeared that the data at the lowest temperatures were relatively unaffected by temperature, the model was constrained in that the slope at this temperatures was set to zero. Then, the change point was determined by fitting the regression line with the smallest sum of squares value. This methodology was in keeping with that of Nickerson et al. (1989), and similar to that of Yeager and Ultsch (1989) except that the two lines were constrained to intersect at the change point. The best fit of the data below 12°C was a two-line model with a change point at 6.4°C. The slope between 6.4-12°C yielded a  $Q_{10}$  value of 8.5. This was calculated by multiplying the slope value by 10 (because the  $Q_{10}$  value represents a 10-degree difference) and raising this value to  $e^x$ . The multiple regression model of the form:

$$\log_e Y_i = \alpha_0 + \alpha_1(T_i - T_o)I_{T_o}(T_i) + \alpha_2 \log_e W_i$$

was fit to the data where:

$Y_i$  = oxygen consumption rate

$W_i$  = weight

$T_i$  = temperature

$$T_o = 6.4^{\circ}\text{C}$$

$$I_{T_o}(T_i) = 0 \text{ if } T_i \leq T_o \\ = 1 \text{ if } T_i > T_o$$

The estimated coefficients of this model were:

$$\hat{\alpha}_0 = -2.11 \text{ (y-intercept)}$$

$$\hat{\alpha}_1 = 0.21 \text{ (slope)}$$

$$\hat{\alpha}_2 = 0.5 \text{ (weight coefficient)}$$

$$\hat{T}_0 = 6.4 \text{ (change point)}$$

Thus, oxygen consumption stabilizes below 6.4°C and increases with a  $Q_{10}$  value of 8.5 between 6.4-12°C. Figure 3.2 is a plot of the mean oxygen consumption rate ( $\log_e$ ) of each fish ( $n=35$ ) over the course of experiments conducted below 12°C. The  $r^2$  value was 0.85.

To calculate a  $Q_{10}$  value for high temperatures, a regression line was fit for the data collected between 12-22°C. Nineteen fish were used to fit the following model:

$$\log_e Y_i = -1.97 + 0.112(T_i) + 0.36 \log_e W_i$$

The  $Q_{10}$  value between 12-22°C was 2.4. The data used in these analyses are shown in Table 3.1.

Using the weight-correction factor generated by the multiple regression model ( $0.57 \log W_i$ ), the raw oxygen consumption rate data were converted into  $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$

therefore results from individual fish could be compared to each other (Table 3.1, Figure 3.2) and to literature values. [Note that the model was fit using only the temperatures below 12°C, and some of the data in Table 3.1 were collected at higher temperatures.] There is about a 10-fold difference between the rates at low temperature and those at high temperature. The results are also presented as  $\text{mg O}_2 \cdot 35 \text{ g}^{-1} \cdot \text{h}^{-1}$  because the mean weight of the fish was 35 g; one kilogram is a much greater weight than the heaviest fish (70 g) and is therefore well out of the range of the data used for the model.

## DISCUSSION

Based on the  $Q_{10}$  value calculated between 6.4-12°C (8.5), it can be concluded that cunner hibernate. This large metabolic decrease is not strictly a result of temperature. There is a physiological change occurring in cunner. Below 6.4°C there is little effect of temperature on metabolic rate because cunner are hibernating. This is substantiated by behavioral observations by other investigators who noted that cunner become behaviorally torpid at about 5°C. Other fishes that have high  $Q_{10}$  values at low temperatures are *Micropterus salmoides* and *Anguilla rostrata* (Lemons and Crawshaw, 1981; Walsh et al., 1983). The  $Q_{10}$  value above 12°C (2.4) was within the range of  $Q_{10}$  values (2-3) expected based on the effect of temperature of metabolic rate.

The multiple regression model was a more accurate method of determining the effects of temperature and weight on metabolic rate. The reason for this is two-fold. Other researchers may accept a standard value for  $b = 0.75-0.8$  in the equation  $Q = aW^b$ ,

the model that describes metabolic rate as a function of temperature [discussed in Chapter 2]. This relationship may not hold for all species. Analysis from the present study yielded a value of 0.57. Also, the effect of temperature is not linear and the multiple regression model takes this into account.

When the two-phased model was not constrained to have a zero slope below the change point, similar results were found. The slope of the line at temperatures below the change point (4.8°C) was close to zero, but was actually negative (-0.174). The slope of the line between 4.8-12°C yielded a  $Q_{10}$  value of 5.53. The multiple regression equation used to fit the data (with the same symbols as previously) was:

$$\log Y_i = -1.41 - 0.174T_i + 0.345(T_i - 4.8)I_{T_0}(T_i) + 0.556\log W_i$$

Regardless of how the regression model was fit, the interpretation of the results remains the same except for a small difference in the change point value. The  $Q_{10}$  value increased from 5.5 to 8.5 with the constrained model, but both results strongly suggest that cunner hibernate. The slightly negative slope for the line fit at the colder temperatures in the unconstrained model may suggest that the constrained model may have greater biological significance.

As most research on hibernation (and torpor) in animals has been performed on mammals, it is of interest to compare hibernation in mammals and ectotherms. Unlike ectotherms, however, body temperature in mammals must be maintained to some extent in cold weather when food supplies may be scarce (McFarland et al., 1979). Torpor in mammals is thought to increase chances of survival during periods of severe weather

including low temperatures by providing energy savings (Howard, 1951; Vogt and Lynch, 1982). Torpor is thought to be induced by short days, food restrictions, and temperature changes (Hill, 1975; Lynch et al., 1978; Tannenbaum and Pivorun, 1988). In ground squirrels, the length of the torpid period increased (and body temperature decreased) as ambient temperature decreased (Geiser and Kenagy, 1988). Duration of torpor bouts are dependent upon body and ambient temperature, depletion of energy reserves, and the buildup of metabolic products, and therefore are directly related to metabolic rate (Geiser and Kenagy, 1988). Geiser and Kenagy (1988) speculated that as body temperature decreases, neural sensitivity to metabolite buildup may be reduced, allowing prolonged torpidity. Tannenbaum and Pivorun (1988) suggested that mice living in the more stressful montane environment may have greater selection pressure to use torpor as a survival mechanism to counter starvation and low temperatures than mice in low altitude areas. Because small rodents have a large surface area to volume ratio, they require more food in proportion to body size than larger mammals in order to maintain body temperature (Howard, 1951). Therefore, their small body size may preclude long-term hibernation, migration, or accumulation of massive lipid reserves (Lyman et al., 1982; Tannenbaum and Pivorun, 1988).

The amount of energy savings (or metabolic reduction) was determined in several studies. Characteristically, metabolic rate may be only one twentieth of the rate at normal body temperatures in mammals (McFarland et al., 1979). In pygmy possums, oxygen consumption rates were only 1% of the consumption rates of normothermic animals



(Geiser, 1987). Johansen and Krog (1959) found that oxygen consumption was reduced 25-30 times in hibernating birchmice. The energy savings in *Peromyscus leucopus* in daily torpor amounted to 440-1900 cal (Hill, 1975).

There are several parallels between mammalian sleep and hibernation. Some authors consider torpor to be on a metabolic continuum with sleep in terms of metabolic reduction and energy savings (Walker et al., 1983). Both are periods of reduced activity. Dormant animals have a lower metabolic rate than their active counterparts. But it is not known to what extent metabolic inhibition, reduced food intake, inactivity, and temperature play in reducing metabolism (Crawshaw, 1984). There is also a similarity in terms of temperature regulation. Walker et al. (1977) noted that thermoregulatory adjustments that occur during slow-wave sleep (SWS) are extended into hibernation. It is during SWS, not paradoxical sleep (REM) that the hypothalamus is still operative in controlling body temperature, albeit at a lower temperature (Glotzbach and Heller, 1976). Hence, the fact that hibernation may be an extension of SWS (not PS) ensures that thermoregulation still occurs. Therefore, it is not surprising that the amount of sleeping time spent in SWS, as opposed to paradoxical sleep, increases at low ambient temperatures (Glotzbach and Heller, 1976; Walker et al., 1979; Walker et al., 1983). Because both periods involve a controlled reduction of body temperature they are thought to be time periods of significant energy savings. Another indication of the close ties between the two functions is that hibernation is entered through sleep in ground squirrels and doves (Walker et al., 1977; Walker et al., 1983). During hibernation, as in sleep, respiration is slow and irregular

(McFarland et al., 1979). Crawshaw (1984) noted a relationship between sleep and cold-water dormancy in fish. The bass *Microperus salmoides* exhibited arrhythmic breathing at low water temperatures (Crawshaw, 1984). Because hibernation is a period of controlled body temperature, it is an adaptation of the mammalian thermoregulatory system and not a vestige of poikilothermic processes where there are virtually no endothermic regulations (Walker et al., 1977).

There is evidence of marked energy savings in ectotherms as well as mammals. Calculations by Tsuji (1988) on data by Bennett and Nagy (1977) show that the energy saved during long winter dormancy in the lizard *Sceloporus occidentalis* is equivalent to the amount of energy required to produce one egg, function for two days, or forage for nine days. Energy savings in mammals were described above. The relationship between sleep and hibernation may be important in both groups. Many behavioral and physiological characteristics of sleep and hibernation are similar, such as inactivity and arrhythmic breathing. Sleep as a necessary precursor to the hibernating state was discussed above, and diel changes in cunner activity and metabolism during hibernation will be discussed in Chapter 5.

Table 3.1  
Change in Mean Oxygen Consumption Rate with Temperature

TEMP (°C)	FISH WEIGHT (g)	OXYGEN CONSUMPTION* (mg O <sub>2</sub> ·kg <sup>-1</sup> ·h <sup>-1</sup> )	OXYGEN CONSUMPTION* (mg O <sub>2</sub> ·35g <sup>-1</sup> ·h <sup>-1</sup> )	SAMPLE SIZE (DAYS)
2.5	29.5	6.0 (4.3, 7.7)	0.9 (0.6, 1.2)	8
2.5	18.3	7.4 (5.4, 9.4)	1.1 (0.8, 1.4)	8
2.5	37.9	6.7 (6.1, 7.3)	1.0 (0.9, 1.1)	9
3.6	19.8	7.3 (5.3, 9.2)	1.1 (0.8, 1.4)	5
3.6	24.8	7.1 (5.7, 8.5)	1.1 (0.9, 1.3)	5
3.6	12.8	9.4 (6.3, 12.6)	1.4 (1.0, 1.9)	4
4.3	13.3	6.3 (4.1, 8.5)	1.0 (0.6, 1.3)	7
4.3	27.6	5.2 (3.8, 6.5)	0.8 (0.6, 1.0)	8
4.3	19.5	4.2 (1.9, 6.5)	0.6 (0.3, 1.0)	8
4.4	35.0	5.4 (5.1, 5.6)	0.8 (0.8, 0.9)	15
4.4	18.4	5.4 (5.0, 5.8)	0.8 (0.8, 0.9)	14
4.6	17.8	4.2 (3.9, 4.6)	0.6 (0.6, 0.7)	8
5.2	36.8	6.7 (6.3, 7.1)	1.0 (1.0, 1.1)	12
5.4	35.0	5.2 (4.8, 5.6)	0.8 (0.7, 0.9)	7
5.4	27.1	5.7 (5.4, 6.0)	0.9 (0.8, 0.9)	8
6.5	28.0	5.9 (5.6, 6.2)	0.9 (0.9, 0.9)	19
6.6	31.4	5.5 (5.4, 5.6)	0.8 (0.8, 0.9)	16
6.9	70.7	6.2 (5.0, 7.4)	1.0 (0.8, 1.1)	6
6.9	61.9	6.9 (5.4, 8.4)	1.1 (0.8, 1.3)	7
7.8	20.9	8.7 (7.2, 10.3)	1.3 (1.1, 1.6)	5
7.8	25.2	7.2 (5.1, 9.4)	1.1 (0.8, 1.4)	5
7.8	13.3	9.3 (6.2, 12.4)	1.4 (1.0, 1.9)	5
8.2	62.5	9.4 (9.3, 9.5)	1.4 (1.4, 1.4)	30
8.4	22.4	15.0 (8.8, 21.3)	2.3 (1.3, 3.3)	4

TEMP (°C)	FISH WEIGHT (g)	OXYGEN CONSUMPTION* (mg O <sub>2</sub> ·kg <sup>-1</sup> ·h <sup>-1</sup> )	OXYGEN CONSUMPTION* (mg O <sub>2</sub> ·35g <sup>-1</sup> ·h <sup>-1</sup> )	SAMPLE SIZE (DAYS)
8.4	13.1	9.7 (4.0, 15.5)	1.5 (0.6, 2.4)	3
8.4	9.6	7.3 (2.0, 12.6)	1.1 (0.3, 1.9)	4
8.7	70.7	10.0 (9.6, 10.4)	1.5 (1.5, 1.6)	10
8.7	61.9	11.0 (10.7, 11.3)	1.7 (1.6, 1.7)	10
8.8	76.3	10.1 (9.9, 10.2)	1.5 (1.5, 1.6)	44
8.9	96.2	9.5 (8.6, 10.3)	1.4 (1.3, 1.6)	10
8.9	53.5	10.9 (10.6, 11.2)	1.7 (1.6, 1.7)	10
8.9	27.0	11.9 (9.6, 14.1)	1.8 (1.5, 2.2)	8
8.9	17.0	8.1 (6.8, 9.5)	1.2 (1.0, 1.4)	9
8.9	36.6	10.1 (8.1, 12.1)	1.6 (1.2, 1.9)	9
9.6	10.7	9.6 (9.4, 9.9)	1.5 (1.4, 1.5)	23
13.4	76.3	9.7 (5.2, 14.3)	1.5 (0.8, 2.2)	6
13.4	62.5	15.4 (12.6, 18.1)	2.4 (1.9, 2.8)	6
13.4	17.8	15.7 (10.0, 21.3)	2.4 (1.5, 3.3)	6
14.8	15.9	24.8 (22.6, 27.0)	3.8 (3.5, 4.1)	6
14.8	26.6	19.0 (17.5, 20.5)	2.9 (2.7, 3.1)	8
15.0	11.2	23.4 (22.4, 24.4)	3.6 (3.4, 3.7)	7
16.6	25.2	24.7 (22.0, 27.5)	3.8 (3.4, 4.2)	13
16.6	27.3	20.7 (16.8, 24.5)	3.2 (2.6, 3.7)	12
16.6	41.3	20.7 (18.0, 23.4)	3.2 (2.8, 3.6)	13
18.8	37.4	28.7 (27.7, 29.7)	4.4 (4.2, 4.5)	10
19.0	52.1	21.9 (20.3, 23.4)	3.3 (3.1, 3.6)	6
19.5	16.8	26.5 (21.5, 31.5)	4.0 (3.3, 4.8)	6
19.6	22.4	34.8 (32.2, 37.4)	5.3 (4.9, 5.7)	7
22.4	49.3	37.8 (35.4, 40.2)	5.8 (5.4, 6.2)	10
22.6	38.9	31.8 (29.0, 34.5)	4.9 (4.4, 5.3)	10

TEMP (°C)	FISH WEIGHT (g)	OXYGEN CONSUMPTION* (mg O <sub>2</sub> ·kg <sup>-1</sup> ·h <sup>-1</sup> )	OXYGEN CONSUMPTION* (mg O <sub>2</sub> ·35g <sup>-1</sup> ·h <sup>-1</sup> )	SAMPLE SIZE (DAYS)
22.9	49.1	35.4 (25.6, 45.3)	5.4 (3.9, 6.9)	4
22.9	67.4	56.6 (45.2, 68.0)	8.7 (7.0, 10.4)	4
22.9	38.9	45.6 (40.5, 50.7)	7.0 (6.2, 7.8)	4
22.9	38.9	40.2 (34.6, 45.7)	6.1 (5.3, 7.0)	4

\* Mean values are given with 95% confidence limits in parentheses.

Table 3.1

The mean daily oxygen consumption rates for fifty-four fish are presented both as mg O<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup> and also as mg O<sub>2</sub>·35 g<sup>-1</sup>·h<sup>-1</sup> (mean weight is 35 g). The temperatures at which the values were collected are listed as well as the fish weight and the number of days the fish were used. The 95% confidence intervals are also presented. Data collected below 12°C are plotted in Figure 3.2.

Figure 3.1

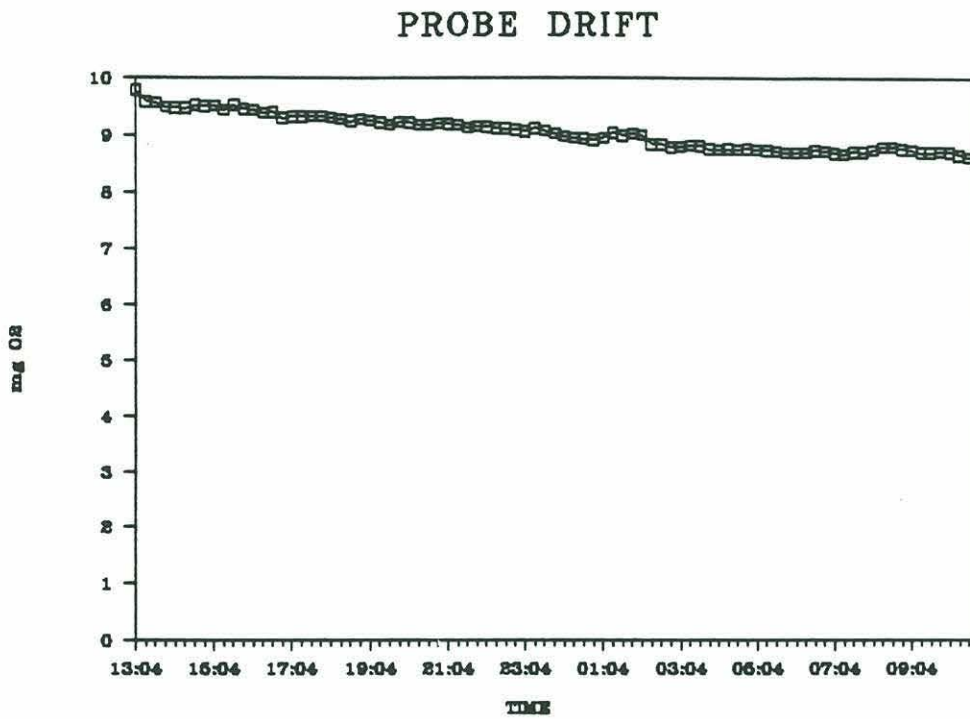


Figure 3.1

The oxygen electrodes experienced drift throughout the period in which they were used and they were recalibrated every day. The rate of change of drift was assumed to be linear. Subsequently, to determine fish oxygen consumption rates, each time point was corrected by the appropriate amount of drift.

Figure 3.2

Oxygen Consumption Rates ( $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ )  
as a Function of Temperature

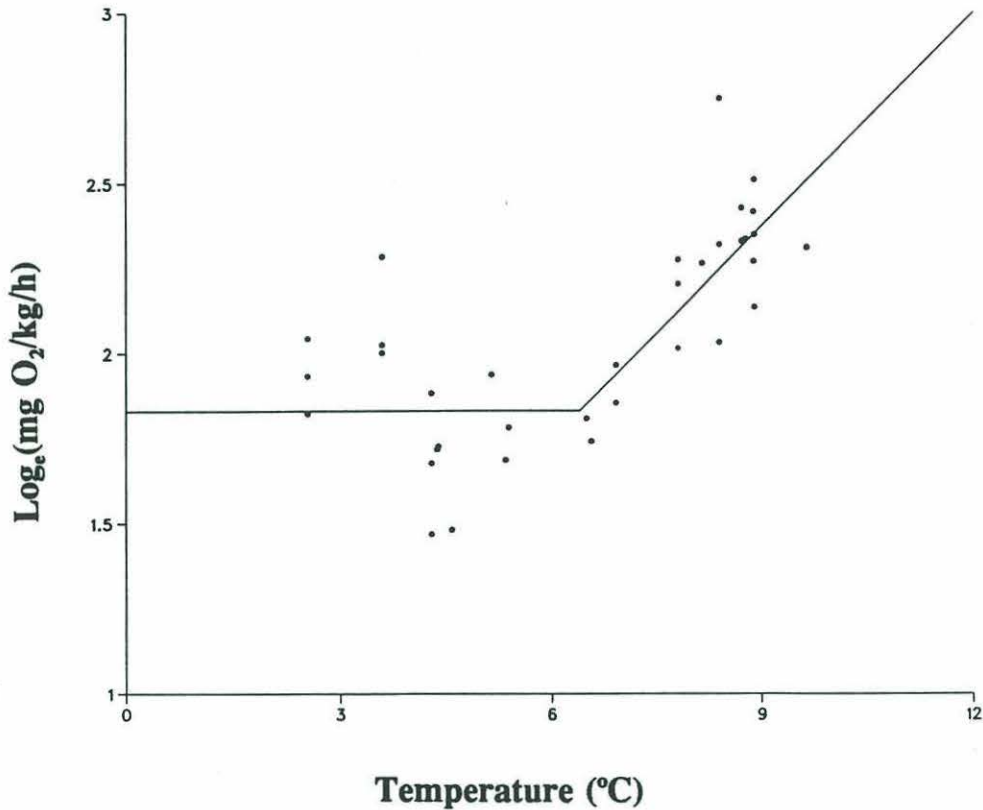


Figure 3.2

A two-phase regression model was fit to data representing the mean oxygen consumption rate for 35 fish used at temperatures below 12°C. The change point occurred at 6.8°C, below which the model was constrained to have a slope of zero. Between 6.8-12°C the slope corresponded to a  $Q_{10}$  value of 8.5. The  $r^2$  value was 0.85. The actual data values (the mean of the daily averages for each fish) and sample sizes (number of days) are listed in Table 3.1.





## CHAPTER 4

### THE EFFECT OF COLD-WATER STARVATION IN THE CUNNER *TAUTOGOLABRUS ADSPERSUS* (WALBAUM) 1792

#### INTRODUCTION

Fishes are known for their ability to undergo prolonged periods of starvation. In fact, many species withstand predictable seasonal periods of food deprivation, particularly in the winter (Love, 1970). Some of the most remarkable periods of food-deprivation have been cited for the eel *Anguilla anguilla*. They can live up to four years and 134 days without eating and can lose 76% of their body weight (Boetius and Boetius, 1967; Boetius and Boetius, 1985). An estivating fish such as *Protopterus* can live three and a half years without eating (Smith, 1935). Smallwood (1916) noted that a female *Amia calva* lived twenty months without eating. The hagfish *Eptatretus stouti* can be kept in the laboratory unfed for 14 months (Inui et al., 1978).

Fishes may have a better mechanism to cope with starvation than mammals, because the former normally consume a large amount of dietary protein (Love, 1970). Unlike mammals, which use glycogen first, fishes may expend their body protein as an energy source during the early stages of starvation. Therefore the effect of starvation on fishes may be less severe (Renaud and Moon, 1980a; Shimeno et al., 1990).

Starvation does not affect all organs to the same extent in fishes. In general, liver and muscle are affected first, while other organs such as the brain remain unaffected (Love, 1970). In *Salmo gairdneri*, reproductive organs decreased in size and females had fewer

eggs but of normal size (Scott, 1962). Similarly in herring, egg production was inversely related to the fat content of the female (Anokhina, 1959).

The three main energy sources for fishes are glycogen, lipid, and protein; the importance of each and the order in which they are mobilized varies among species. Love (1970) noted the general order of usage as glycogen, lipid, then protein, the latter being used only after the former two are depleted, but other authors have since found that the depletion of reserves may not follow this order (Creach and Serfaty, 1974; Leger, 1981; Jezierska et al., 1982; Lewis and Epple, 1984; Takeuchi et al., 1987; Machado et al., 1988). This study was designed to investigate the effects of food deprivation on glycogen, lipid, and protein stores in the cunner, which undergoes long periods of starvation during hibernation. Also, a predictive model for the potential maximum time that this species can survive without eating was formulated.

## MATERIALS AND METHODS

In order to determine changes in both liver and whole-body values of protein, lipid, and glycogen levels over a prolonged period of starvation, 40 fish were maintained in a 4°C cold room. They were randomly assigned to ten 12-liter containers that were divided into quarters. The fish were randomly sacrificed at monthly intervals by over-anaesthetizing them using carbon dioxide. Fish were kept on ice for as much as several hours until each of the livers were removed and the rest of the body was homogenized following the comparative slaughter technique described by McDonald et al. (1973). The sex of the fish was determined by visual inspection in fish with developed gonads.

## **Dry Weight**

Approximately 100 mg of fish homogenate and 40 mg of liver were used to determine the percent dry weight of these two components for each individual fish. Samples were placed in a drying oven at 60°C for 48 h following the procedure of Leavitt et al. (1990).

## **Lipid Analysis**

Approximately 25 mg of liver or body sample was homogenized and the lipid was extracted using a chloroform:methanol mixture following a protocol modified from Bligh and Dyer (1959). In brief, 1.5 ml of chloroform:methanol solution in a 1:2 ratio was added to the sample along with 400  $\mu$ l of water. After allowing the samples to stand for 10-15 minutes, 1.5 ml of chloroform:methanol mixture in a ratio of 2:1 was added and again the samples stood for 10-15 minutes with intermittent mixing. By using two different ratios, lipids with different polarities were maximally extracted. As described by Folch et al. (1957), in order for any acidic lipids in the upper-bilayer to become part of the bottom layer, 0.95 ml of 0.7% NaCl solution was added. After 30 min in the refrigerator (6°C), samples were centrifuged briefly (less than a minute), the volume of the bottom layer was recorded, and the upper layer containing the methanol and any interfacial fluff was removed. Twenty-five  $\mu$ l of each sample was placed on pre-weighed pans. After evaporation, the samples were weighed on a Cahn balance to  $10^{-4}$  mg. Using the weight of the sample, the volume transferred, and the total volume of the lipid layer, the lipid concentration per sample could be calculated.

## Protein Analysis

Protein concentrations were determined from the same samples used for the lipid extractions using modified techniques described by Lowry et al. (1951) and Raymont et al. (1964). The protein was resuspended in a mixture of water, and a biuret solution was used as a coloring agent. One of the advantages of the biuret method over other reactions is that the coloration is more constant (Lowry et al., 1951). A standard curve relating spectrophotometer absorbance with protein concentration was generated using a range of Bovine Serum Albumin (BSA) concentrations. The equation generated was:

$$\text{Protein (mg)} = \frac{\text{Absorbance} - 0.019}{0.048}$$

The  $r^2$  value was 0.99. Samples were read in duplicate on a spectrophotometer at a wavelength of 540 nm. Protein values are presented in mg protein/mg body dry weight, wet weight, and as a fraction of the wet or dry liver weight for the liver samples.

## Glycogen Analysis

Approximately 10-15 mg of liver tissue and 50-60 mg of body homogenate were used for the glycogen analyses. Samples were kept frozen at  $-70^{\circ}\text{C}$  until processed using a modified version of the colorimetric Sigma analysis (kit 510) and the technique described by Roehrig and Allred (1974). Glycogen enzymatic hydrolysis is preferable to acid hydrolysis (Keppler and Decker, 1974) because it is faster than acid hydrolysis and less apt to cause any glycogen loss. Samples were homogenized, and 0.4 ml of buffer was added with 0.1 ml amyloglucosidase (from *Aspergillus niger*), which degrades glycogen to glucose as described by Keppler and Decker (1974). Samples were incubated for 2 h at  $37^{\circ}\text{C}$ . A 0.5 ml portion of the sample was added to 5 ml of the enzyme-color reagent

solution. This solution was a mixture of glucose oxidase, peroxidase, and buffer salts plus o-dianisidine dihydrochloride. The samples were allowed to develop in the dark for 1 h and 10 min. Absorbance was determined photometrically and read on the spectrophotometer. A standard curve was generated to relate absorbance to glycogen content. Samples were read at 440 nm in duplicate. Glycogen content was calculated using the following formula:

$$\text{Glycogen (ug/100 ul)} = \frac{\text{Absorbance} + 0.02499}{0.00045}$$

The  $r^2$  value was 0.99.

## RESULTS

The mean percent dry weights for the monthly lipid and protein samples ( $\pm 1$  SE) are presented in Tables 4.1 and 4.2. Percent dry weight, as opposed to wet weight, was used because it eliminates the variability due to water content. The glycogen concentration (ug/mg) for each month ( $\pm 1$  SE) is presented in Table 4.3. The results are also presented in figure format and will be discussed below.

Changes in energy stores over time are illustrated in the following histograms. To determine significant differences ( $p < 0.5$ ) among the monthly sampling periods, Tukey's multiple comparison test was used. Those values presented as percentages were log transformed after adding 1 to each data point (Zar, 1984). There was no significant difference in muscle glycogen, lipid, or protein values over time (Figs. 4.1, 4.2, 4.3,

respectively). For liver glycogen, January was significantly higher than April (Fig 4.4). Note that the December values for both body glycogen and liver glycogen were lower than the January values (Figs. 4.1 and 4.4). This was caused by an error in sampling protocol that was corrected in later months. The December samples probably remained at room temperature too long causing the glycogen to convert into lactic acid. Therefore, the December values were omitted from all glycogen figures. For percent liver lipid, December was significantly higher than February (Fig. 4.2). For percent liver protein (Fig. 4.3), January and February were significantly lower than April.

The results are also presented in terms of total component lost per standard fish. The average weight of the 40 fish was 20.6 g, therefore a weight of 20 g was used for the "standard" fish. Because no significant differences were observed for the body homogenate data, only the liver data were analyzed in this fashion. All three components decreased with time. Glycogen values (Fig. 4.5) for January were significantly higher than April and March (recall the methodological error in the December glycogen samples). By performing a regression analysis on the four remaining months, the glycogen value in December was extrapolated to be 425 mg. For lipid (Fig. 4.6), December was significantly different from March and April. Protein values in December were significantly different from March and April, and January was significantly different from March (Fig. 4.7), again demonstrating the decrease in an energy store over time.

## ENERGY BUDGET

Given the amounts of glycogen, lipid, and protein used, the contribution of each of these components to the energy budget of the cunner can be derived. The conversion factors used for carbohydrate, lipid, and protein were  $23.6 \text{ J}\cdot\text{mg}^{-1}$ ,  $39.5 \text{ J}\cdot\text{mg}^{-1}$ , and  $16.7 \text{ J}\cdot\text{mg}^{-1}$ , respectively. The energy yielded per month for glycogen, lipid, and protein were  $120 \text{ J}\cdot\text{mg}^{-1}$  ( $300 \text{ cal}\cdot\text{g}^{-1}$ ),  $150 \text{ J}\cdot\text{mg}^{-1}$  ( $400 \text{ cal}\cdot\text{g}^{-1}$ ), and  $120 \text{ J}\cdot\text{mg}^{-1}$  ( $300 \text{ cal}\cdot\text{g}^{-1}$ ), respectively.

There was no significant difference in the weight or standard length of the eight fish sacrificed for each of the monthly time periods ( $p < 0.05$ ). There was no significant difference in the percent water content of the liver over time ( $p < 0.05$ ), but there was a significant difference in the percent water content of the body over time. Using Tukey's multiple comparison test, January was significantly higher than March and April indicating that water content was increasing in the muscle. Analyzed as percent of total body wet weight, the liver weight decreased significantly with time; December and January were both higher than March or April (Fig. 4.8).

## DISCUSSION

Of all the energy stores, carbohydrates, such as glycogen, are the most readily utilized, and for most vertebrates the main storage site is the liver (Hochachka and Somero, 1984). Glycogen in the liver is mobilized to replace muscle glycogen but must be broken down to glucose during transport by the blood and may be used as glucose in the muscle, or reconverted into glycogen for later use. Rapid glycogen mobilization has

been noted by several authors (Inui and Ohshima, 1966; Love, 1970; Mahajan and Dheer, 1983; Black and Love, 1986).

Other fish species obtain most of their glycogen supply from muscle stores. In the hagfish *Eptatretus stouti*, the liver has a smaller proportion of glycogen than the body, which is unusual when compared to other vertebrates (Inui and Gorbman, 1977; Inui and Gorbman, 1978); the liver glycogen concentration was only 25% of that of muscle (Inui et al., 1978). Because the relative muscle mass is much greater than liver mass, a larger amount of available glycogen is found in the muscle tissue (Inui et al., 1978). In the hagfish *Myxine glutinosa*, skeletal muscle glycogen was the primary source of energy (Emdin, 1982). Over 90% of the glycogen in the liver and skeletal muscle was consumed, but protein and triglyceride contents did not undergo much change during starvation (Emdin, 1982). Also, in the mudskipper *Boleophthalmus boddarti*, muscle glycogen, not liver glycogen, was utilized preferentially (Lim and Ip, 1989). In the eel *Anguilla japonica* and the catfish, muscle glycogen decreased (Inui and Ohshima, 1966; Malhotra and Sharma, 1981).

A difficulty in estimating the amount of carbohydrates mobilized during starvation is that gluconeogenesis may occur (Machado et al., 1988; Heming and Paleczny, 1987). Food deprivation stimulates lactate gluconeogenesis in fishes (Renaud and Moon, 1980b). Therefore, anaerobic metabolism of glucose is high in fishes and is more important than it is in mammals. Glycogen actually increased or stayed constant during starvation in *Anguilla anguilla*; this was due in part to enhanced lactate gluconeogenesis suggesting that this is the major carbohydrate source (Renaud and Moon, 1980a, 1980b). Glycogen



levels of tissues depend on differences in nutritional state and hormonal levels (Keppler and Decker, 1974; Holopainen and Hyvarinen, 1985). In the crucian carp *Carassius carassius*, a large store of liver glycogen is accumulated in the late summer and fall, and about 80% of it is used during winter fasting.

Liver glycogen is not converted into glucose as readily in fishes as it is in mammals, but there is a great deal of variability among species (Hilton, 1982). Aerobic oxidation and utilization of glucose in fishes is low, even when temperature differences are taken into account (Lin et al., 1978). Therefore, dietary carbohydrates may not be a major source of energy for fishes (Cowey and Sargent, 1979), but dietary lipids may. Mammals are also capable of utilizing glucose more rapidly than fish because they have higher activity levels for the relevant enzymes (Garcia de Frutos et al., 1991; Cowey and Sargent, 1979). Glycogen is the first compound to be depleted during fasting in mammals (Lim and Ip, 1989). Starved fishes oxidize substrates other than glucose before mobilizing and hydrolysing glycogen (Cowey and Sargent, 1979). The presence of a large quantity of oil in fishes indicates that lipids, not carbohydrates, are the favored energy reserve of fishes (Cowey and Sargent, 1979). During starvation in carp, lipid was exhausted from the hepatopancreas, but some glycogen remained. This indicates a difference between mammals and fishes, because in the former, liver glycogen is usually exhausted within 1-2 days of food deprivation followed by lipid consumption (Nagai and Ikeda, 1971).

In some fish species, lipid is the source of metabolic fuel, and it may be depleted to a great extent during starvation (Creach and Serfaty, 1974; Leger, 1981; Jezierska et al.,

1982; Takeuchi et al., 1987; Machado et al., 1988). Lipid may be accumulated prior to periods of reduced food availability (Miglav and Jobling, 1989) and stored under the skin, in the stomach wall, or in the liver or muscle (Tashima and Cahill, 1965; Love, 1970). Wilkins (1967) noted that in *Clupea harengus* stored fat may comprise 30% of the wet weight in July/August, but may comprise only 1% in winter. In the red sea bream *Pagrus major* starvation tolerance was highly correlated with muscle lipid level (Nakagawa et al., 1991).

In many fish species, endogenous protein is metabolized before other reserves are exhausted (Wilkins, 1967; Creach and Serfaty, 1974; Takeuchi et al., 1987; Machado et al., 1988; Shimeno et al., 1990; Bastrop et al., 1991; Stimpson, 1965). This contrasts with mammals, which do not expend protein reserves until all lipid and carbohydrate stores are depleted. Also, in contrast to mammals, protein may even serve as a more important source of energy than glycogen during starvation (Creach and Serfaty, 1974; Lewis and Epple, 1984; Machado et al., 1988). Changes in liver and muscle (or body) stores during starvation in several species (including cunner results from this study) are shown in Table 4.4 and Table 4.5, respectively. The data are shown as percent wet weight of liver (or body/muscle), and also as the percent remaining of the initial stores.

There is an inverse relationship between lipid content and water content in muscle. As lipid is lost during starvation, body weight is initially maintained through water intake (Templeman and Andrews, 1956; Wilkins, 1967; Love, 1970; Lewis and Epple, 1984; Boetius and Boetius, 1985; Nakagawa and Kasahara, 1986; Takeuchi et al., 1987; Nihlavs and Jobling, 1989). An increase in water concentration also corresponds to a

decrease in white muscle protein. The most dramatic example of water increase was found in the species *Hippoglossoides platessoides*, having a muscle water content of 96%. The protein content was less than 3% and lipid content was 0.06% (Templeman and Andrews, 1956). The degree to which weight loss occurs will in part depend on the maintenance temperature, the initial size, and condition of the fish species under investigation.

The decrease of the liver weight to body weight ratio (often referred to as the Liver Somatic Index) has been documented in many species (Tashima and Cahill, 1965; Inui and Ohshima, 1966; Lewis and Epple, 1984; Pandey and Singh, 1984; Thomas et al., 1986; Miglavs and Jobling, 1989; Shimeno et al., 1990). The results from this study also indicate that there was a significant decrease in the liver/body weight ratio over time (Fig 4.8). Liver glycogen, lipid, and protein all decreased during the experiment, each contributing to the decrease in liver weight.

Of the three energy stores investigated in cunner, all components decreased with increased period of food deprivation, but glycogen values were the most accessible and were used first. Glycogen concentrations initially decreased faster than lipid or protein. The protein concentrations decreased the slowest. Proteins were conserved not only because they are an essential component of the cell matrix, but also because they are a less accessible and less efficient food source than both glycogen and lipid. Given the rate of utilization and the generated regression line, liver glycogen would be exhausted in May. As the lipid and protein levels were still high and, in the case of body constituents, not mobilized to a significant extent, the cunner could remain in a state of food

deprivation for a period much longer than required for overwintering. Based on the rate of decrease of reserves in the liver, cunner could live at least six months on the glycogen and lipid reserves, and nine months on the protein stores. Because these time projections are based solely on liver stores, they are a conservative prediction of survival time, although some lipid and protein are required for structural integrity. There was no significant decrease in body components over time, and the importance of these reserves may be underestimated. Given that most of the body weight is muscle, even relatively small concentrations of reserves may sum to a relatively large energy resource (Machado et al., 1988). The small decrease in body weight during starvation relative to other species (Table 4.6) is further evidence that metabolic rate is low (Chapter 3) and that energy reserves are not completely exhausted. The amount of weight loss was much lower than reported in many other starvation studies (Table 4.6). The low temperature at which the experiment was conducted and the low metabolic rate of cunner may be partly responsible. The results of this study predict that the biogeographic range of cunner is not limited by its ability to withstand food deprivation. The metabolic rate is extremely low and their reserves are used conservatively; they could easily survive without food for many months, possibly a year, in 4°C water in boreal areas.

Table 4.1  
Mean Percent Lipid Concentration

MONTH	LIVER			BODY		
	MEAN	SE	N	MEAN	SE	N
DEC	24.2	2.6	7	12.7	1.8	8
JAN	17.7	2.5	8	11.1	1.1	8
FEB	15.7	1.0	8	10.9	1.0	8
MAR	18.1	1.6	9	10.1	1.2	8
APR	16.0	1.0	8	8.3	0.6	8

Table 4.1

There was no significant difference in body lipid content over time, however, liver lipid content decreased over time. Using Tukey's multiple comparison test, December was significantly higher than February ( $p < 0.05$ ). Note Fig. 4.2.

Table 4.2  
Mean Percent Protein Concentration

LIVER				BODY			
MONTH	MEAN	SE	N		MEAN	SE	N
DEC	56.9	5.0	7		71.6	3.8	8
JAN	49.9	1.1	8		65.3	3.7	8
FEB	50.0	2.0	8		76.9	3.5	8
MAR	53.1	2.7	9		70.5	2.6	8
APR	65.0	3.5	8		71.1	3.2	8

Table 4.2

There was no significant difference in body protein content over time. For liver protein, January and February were significantly lower than April ( $p < 0.05$ ) using Tukey's multiple comparison test. This is potentially an artifact of the decrease in lipid and glycogen content. Note Fig. 4.3.

Table 4.3  
Mean Glycogen Concentration

MONTH	LIVER				BODY		
	MEAN (ug/mg)	SE	N		MEAN (ug/mg)	SE	N
DEC	206	36.0	8		3.38	0.5	8
JAN	377	42.0	8		4.37	0.5	8
FEB	278	30.2	8		4.24	0.6	8
MAR	236	56.4	8		4.28	0.7	8
APR	182	34.9	8		4.22	0.6	8

Table 4.3

There was no significant difference in body glycogen over the course of the experiment. Refer to Fig. 4.1. For liver glycogen, the April value was significantly lower than January ( $p < 0.05$ ) using Tukey's multiple comparison test. Refer to Fig. 4.4. A methodological error affected the December samples, causing unusually low glycogen values to be obtained.

Table 4.4  
The Effect of Starvation on Liver Components

SPECIES	TEMP (°C)	DAYS W/O FOOD	GLYCOGEN % WET WT, AND % OF INITIAL	LIPID % WET WT, AND % OF INITIAL	PROTEIN% WET WT, AND % OF INITIAL	SOURCE
<i>Anguilla japonica</i>	12	95	3.7, 39	3.8, 55		Inui and Ohshima, 1966
<i>Anguilla japonica</i>	28	95	2.9, 52	3, 50		"
<i>Cyprinus carpio</i>	20	101	1.6, 19			Nagai and Ikeda, 1971
<i>Cyprinus carpio</i>	22	28			12, 92	Bastrop et al., 1991
<i>Carassius auratus</i>	24	8	2.0, 54	2.8, 7		Stimpson, 1965
<i>Carassius auratus</i>	37	8	1.6, 28			"
<i>Salvelinus alpinus</i>		112		4.6, 115	13.4, 126	Miglavs and Jobling, 1989
<i>Colisa fasciatus</i>	27	40		1.7, 17		Pandey and Singh, 1984
<i>Tautoglabrus adspersus</i>	4	135	4.2, 37*	3, 50	14, 110	This study

\* = estimate of 37% based on a regression estimate for the initial December value

Table 4.4

The effect of starvation on liver glycogen, lipid, and protein stores are presented as percent wet weight (wt) and also as the percent of the store remaining at the end of each experiment (percent of initial). Glycogen stores are depleted more than that of lipid or protein. In some cases the latter two reserves exhibited an increase in concentration during starvation, possibly as an artifact of the rapid decrease in glycogen.



Table 4.5  
The Effect of Starvation on Muscle Components

FISH	TEMP (°C)	DAYS W/O FOOD	GLYCOGEN % WET WT, AND % OF INITIAL	LIPID % WET WT, AND % OF INITIAL	PROTEIN % WET WT, AND % OF INITIAL	SOURCE AND COMMENTS
<i>Carassius auratus</i>	24	8	0.12, 63	1.65, 138		Stimpson, 1965
"	37	8	0.5, 76			Stimpson, 1965
<i>Cyprinus carpio</i>	22	28			15, 99	Bastrop et al., 1991
<i>Phoxinus phoxinus</i>	5	21		24, 95	49, 104	Cui and Wooton, 1988 dry carcass
"	9	21		20, 86	50, 100	"
"	12	21		13, 70	58, 102	"
"	15	21		16, 68	50, 103	"
<i>Salvelinus alpinus</i>		112		1.6, 33	7.4, 82	Miglavs and Jobling, 1989 carcass
<i>Pagrus major</i>	13-22	27		0.1, 20	15, 79	Nakagawa et al., 1991
<i>Colisa fasciatus</i>	27	40		0.8, 28		Pandey and Singh, 1984 carcass
<i>Tautoglabrus adspersus</i>	4	135	0.1, 83*	2, 61	15, 93	This study body

\* = estimate of 83% based on regression estimate for the initial December value

Table 4.5

The effect of starvation on glycogen, lipid, and protein stores in the muscle (or body/carcass as indicated) is presented. Energy stores are presented as percent wet weight (wt) and also as the percent of the store remaining at the end of each experiment (percent of initial). Glycogen exhibited the most dramatic decrease, and lipid and protein stores decreased to a lesser degree and in some cases increased, possibly as an artifact of the substantial decrease in glycogen.

Table 4.6  
Weight Loss of Fishes Undergoing Starvation

SPECIES	DAYS W/O FOOD	TEMP (°C)	WEIGHT LOSS (WET) AS % INITIAL WEIGHT	MEAN DAILY LOSS (%)	SOURCE
<i>Eptatretus cirrhatus</i>	14	11	11	0.8	Forster, 1990
<i>Anguilla japonica</i>	93	12	19	0.2	Inui and Ohshima, 1966
<i>Anguilla japonica</i>	95	28	36	0.38	Inui and Ohshima, 1966
<i>Clupea harengus</i>	129	6-12	37	0.29	Wilkins, 1967
<i>Cyprinus carpio</i>	30	16-19	14	0.47	Shimeno et al., 1990
<i>Carassius auratus</i> (2-8 g)	12	24	13.4	0.56	Stimpson, 1965
<i>Carassius auratus</i> (60-90 g)	12	24	5.1	0.21	Stimpson, 1965
<i>Salvelinus fontinalis</i>	56	11.4	19	0.34	Heming and Paleczny, 1987
<i>Salmo gairdneri</i>	56	11.4	14	0.25	Heming and Paleczny, 1987
<i>Salmo gairdneri</i>	36	10-12	18	0.50	Leger, 1981
<i>Colisa fasciatus</i>	40	27	27	0.67	Pandey and Singh, 1984

<i>Anoplopoma fimbria</i>	177	6-10	11.2	0.06	Sullivan and Smith, 1982
<i>Lepomis machrochirus</i>	42	21	14	0.33	Kitchell and Windell, 1970
<i>Tautogolabrus adspersus</i>	135	4	10	0.07	This study
<i>Boleophthalmus boddarti</i>	21	-	12	0.57	Lim and Ip, 1989

Table 4.6

The effect of starvation on several fish species over a range of temperatures is presented. The small daily weight loss exhibited by *Tautogolabrus adspersus* may be the result of both the low temperature at which the experiment was conducted, and its relatively low use of energy stores.

Figure 4.1

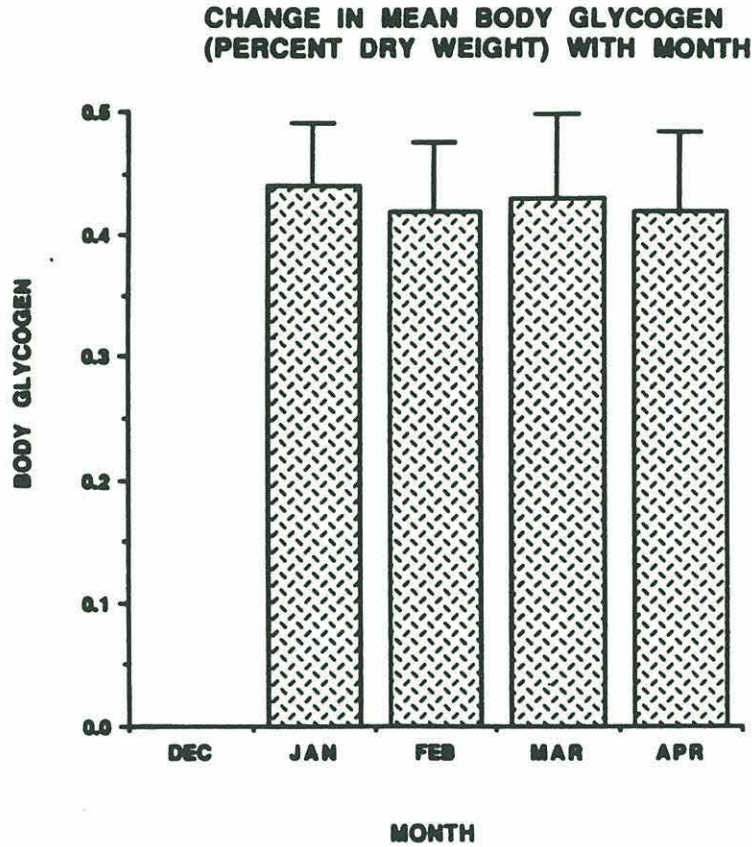


Figure 4.1

There was no significant difference in body glycogen over the course of the experiment. Refer to Table 4.3 for data values. A methodological error affected the December samples, causing unusually low glycogen values to be obtained (see text). Eight individuals were used each month. Error bars are  $\pm 1$  SE.

Figure 4.2

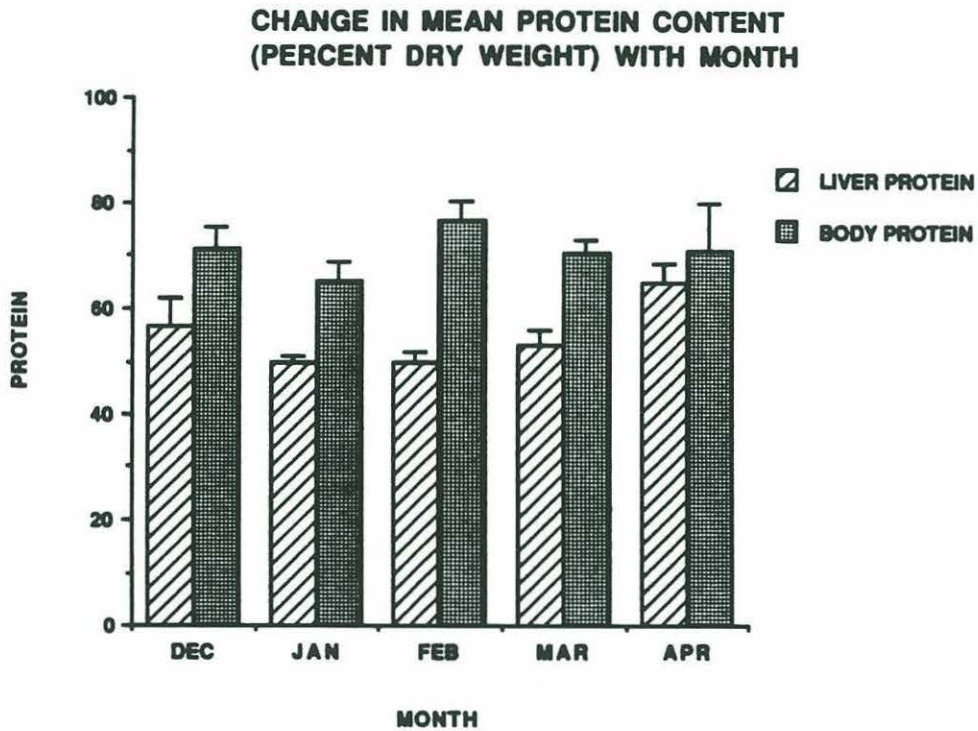


Figure 4.2

There was no significant difference in body lipid content over time. Liver lipid content decreased over time. Using Tukey's multiple comparison test, December was significantly higher than February ( $p < 0.05$ ). Data values and sample sizes are listed in Table 4.1. Error bars are  $\pm 1$  SE.

Figure 4.3

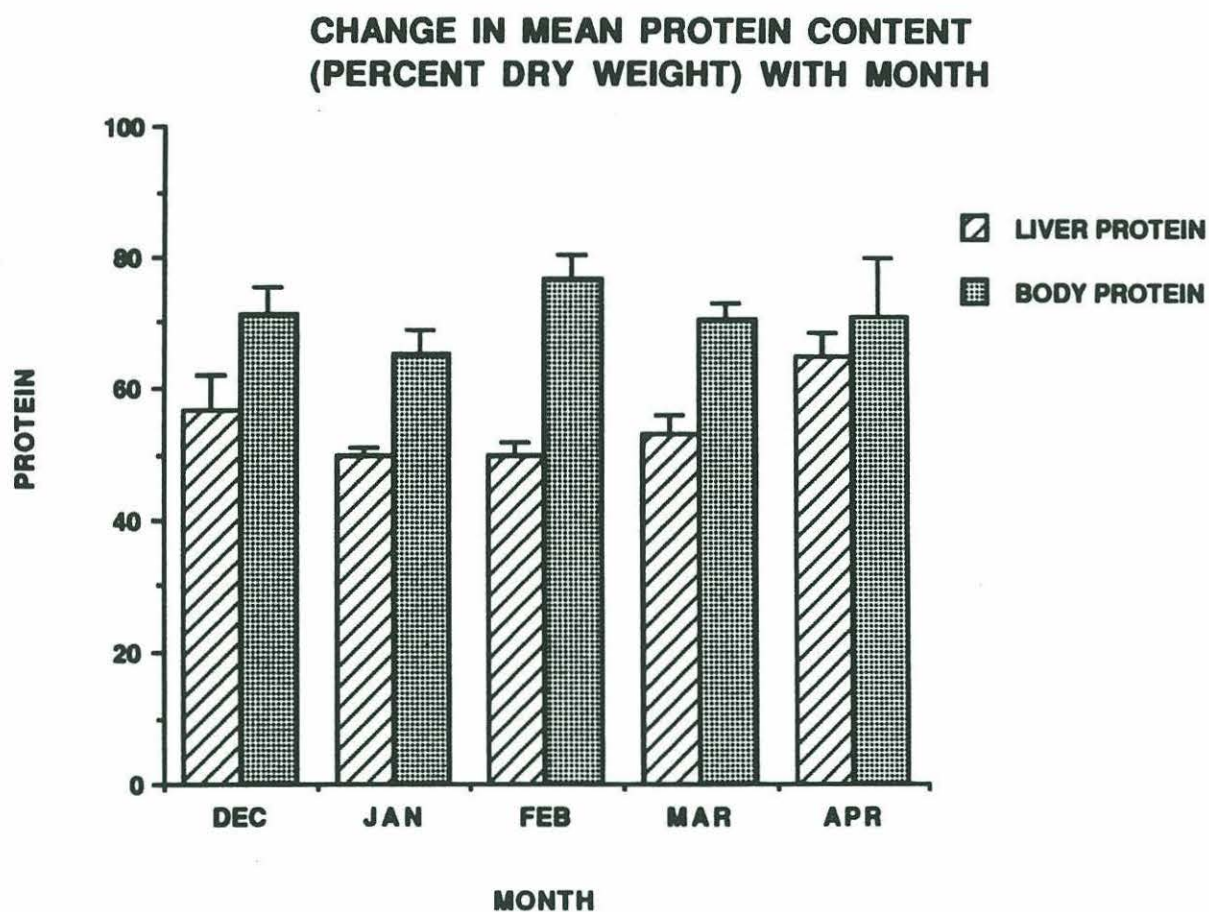


Figure 4.3

There was no significant difference in body protein content over time. For liver protein, January and February were significantly lower than April ( $p < 0.05$ ) using Tukey's multiple comparison test. This may be an artifact of the decrease in lipid and glycogen. Data values and sample sizes are listed in Table 4.2. Error bars are  $\pm 1$  SE.

Figure 4.4

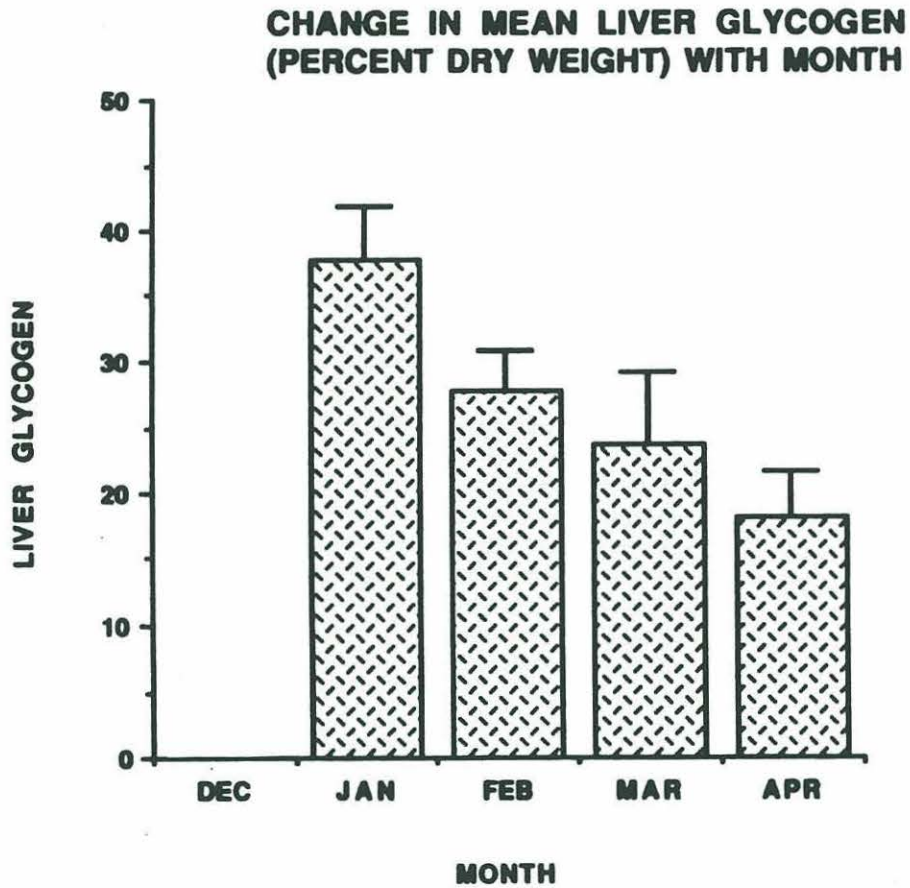


Figure 4.4

The mean liver glycogen value for eight fish for each month is presented. Using Tukey's multiple comparison test, April was significantly different from January ( $p < 0.05$ ). A methodological error affected the December samples, causing unusually low glycogen values to be obtained (see text). Data values are listed in Table 4.3. Error bars are  $\pm 1$  SE.

Figure 4.5

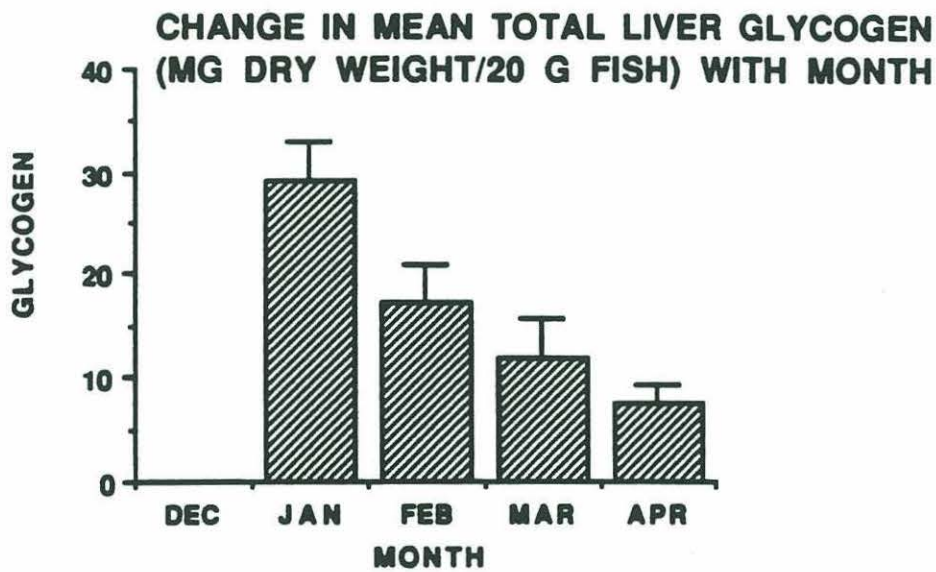


Figure 4.5

The liver glycogen concentrations for each fish were standardized in order to compare values for different-sized fish. As 20 g was the mean weight of the experimental fish, all values were converted to represent the amount of glycogen present in a 20 g fish. Using the Tukey's multiple comparison test, January was significantly greater than March and April ( $p < 0.05$ ). A methodological error affected the December samples, causing unusually low glycogen values to be obtained (see text). Eight individuals were used each month. Error bars are  $\pm 1$  SE.



Figure 4.6

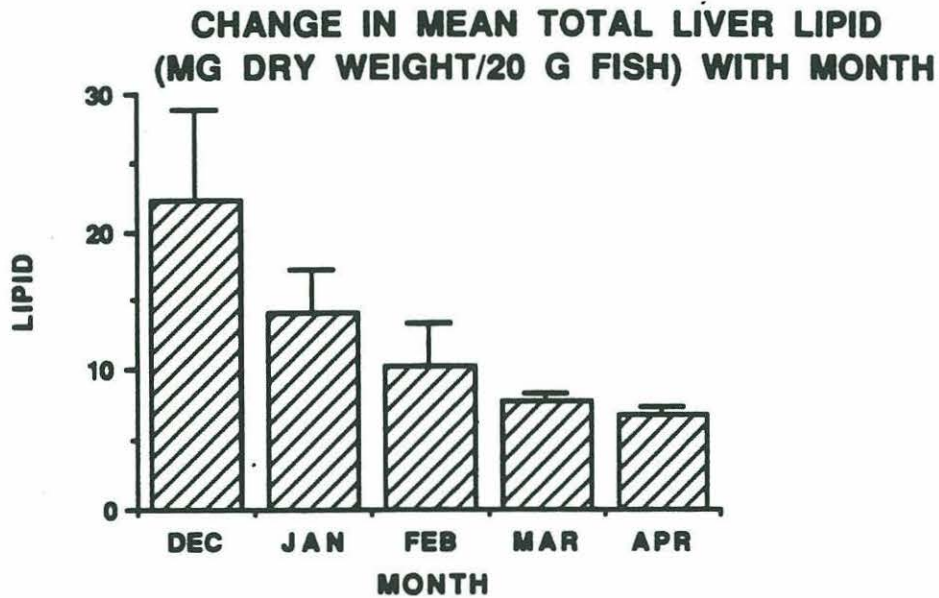


Figure 4.6

The liver lipid concentrations for each fish were standardized in order to compare values for different-sized fish. As 20 g was the mean weight of the experimental fish, all values were converted to represent the amount of lipid present in a 20 g fish. Liver lipid content decreased over the course of the experiment. Using Tukey's multiple comparison test, December was significantly different from March and April, and January was significantly different from April ( $p < 0.05$ ). Sample sizes are listed in Table 4.1. Error bars are  $\pm 1$  SE.

Figure 4.7

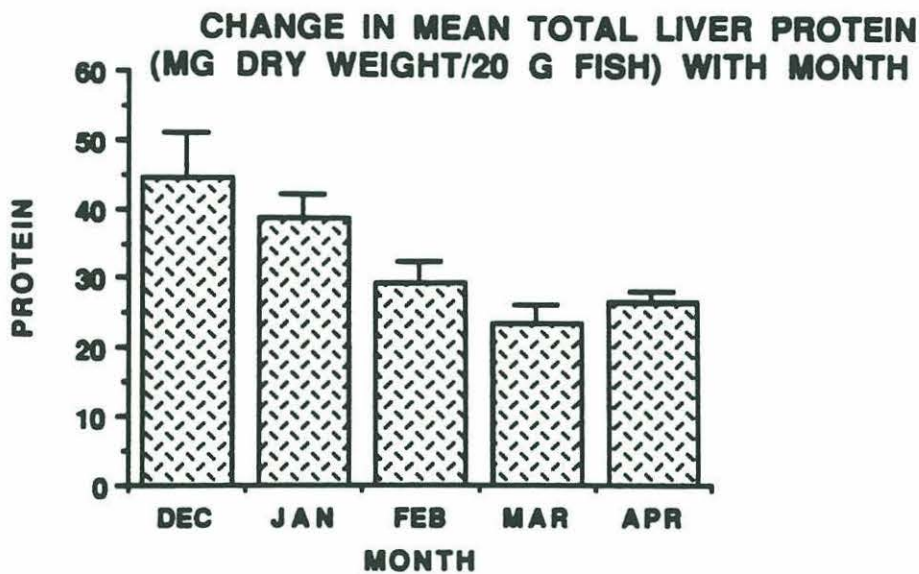


Figure 4.7

The liver protein concentrations for each fish were standardized in order to compare values for different-sized fish. As 20 g was the mean weight of the experimental fish, all values were converted to represent the amount of protein present in a 20 g fish. Liver protein content decreased over the course of the experiment. Using Tukey's multiple comparison test, December was significantly different from March and April ( $p < 0.05$ ). Sample sizes are listed in Table 4.2. Error bars are  $\pm 1$  SE.

Figure 4.8

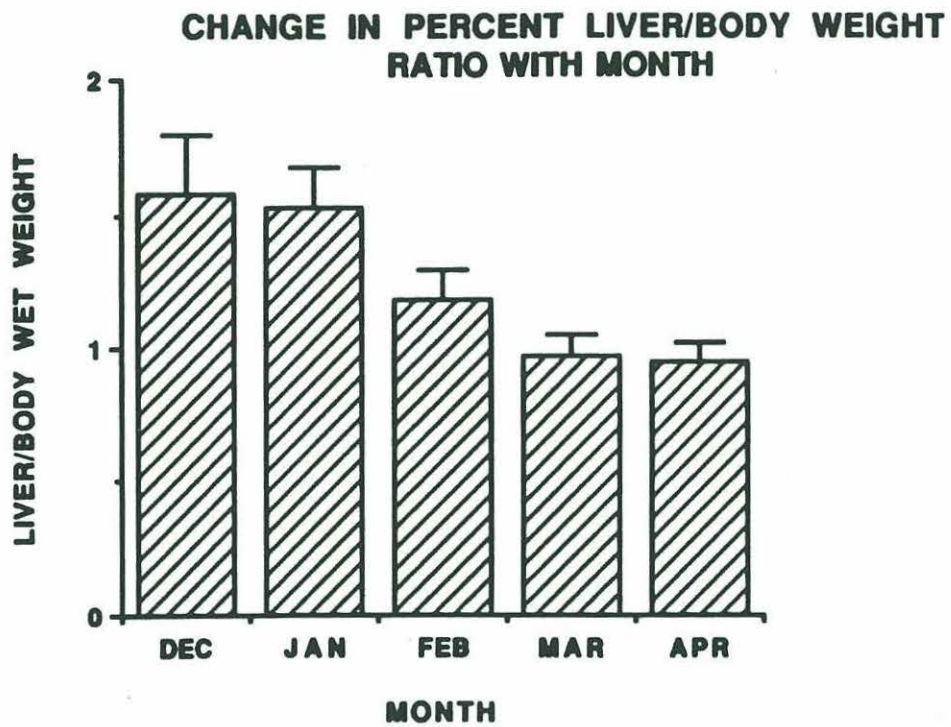


Figure 4.8

The liver/body weight ratio (percent wet weight) decreased with time. Using Tukey's multiple comparison test, December and January were both higher than March or April. Eight individuals were used each month. Error bars are  $\pm 1$  SE



## CHAPTER 5

### DIEL CHANGES IN OXYGEN CONSUMPTION RATES OF THE CUNNER *TAUTOGOLABRUS ADSPERSUS* (WALBAUM) 1792

#### INTRODUCTION

Diel cycles are known to occur in a wide variety of organisms. One of the first reports of circadian rhythm (i.e. an endogenous cycle) in fishes was found in the electric fish *Gymnorhamphichthys hypostomus* by Lissmann and Schwassmann (1965). There was a marked change in activity pattern accompanied by changes in the frequency of discharges for the electric organ. The fact that the cycle continued during periods of constant dim light suggested that the rhythm was endogenous with light being the entraining agent. An endogenous circadian rhythm was also found in egg fanning behavior in the cichlid species *Cichlasoma nigrofasciatum* and *Herotilapia multispinosa* (Reebs and Colgan, 1991). These fishes are diurnal, but during the reproductive period the females become active at night. In the cave-dwelling loach *Oreonectus evezardi*, a circadian rhythm as well as a circannual one was found in its air-gulping behavior (Biswas et al., 1990).

Although many fishes exhibit rhythmic cycles, they are not necessarily circadian rhythms. A circadian rhythm implies that there is an endogenous clock with an inherent sustained periodicity. Instead, the cycles may be driven by external cues such as light. Fishes in the family Serrasalminidae were shown to have a diurnal rhythm in oxygen consumption rates; the rhythm was in response to light and was not an endogenous cycle (Saint-Paul, 1988). A diel cycle in air breathing, locomotion, and feeding was found in

the atipa *Hoplosternum littorale* (Boujard et al., 1990). When the light-dark cycle was shifted, so did these behaviors, indicating that the light cycle was the synchronizer. The oxygen consumption rates of *Lithognathus mormyrus* and *L. lithognathus* also show a diel pattern (Du Preez et al., 1986). Smith and Laver (1981) suggested that a diel rhythm occurred in the bathypelagic fish *Cyclothone acclinidens*; respiration rates at night were 3-5 x higher than during the day. Tobler and Borbely (1985) noted a rest-activity cycle in which the fishes *Cichlosoma nigrofasciatum* and *Carrassius auratus* were more active during the day.

The use of time-series analysis has been an accepted method of analyzing long periods of data on cyclical trends such as diurnal rhythms (Enright, 1965; Thompson, 1982). It is a more accurate way of interpreting the data than breaking up a 24-h period arbitrarily into a day component and a night one and then looking for a repeated pattern. Analysis of periodograms or smooth periodograms (spectral estimates) can determine whether or not there are cyclical trends in data (Enright, 1965). Spectral analysis has been applied to other biological periodicities in fishes, including EEG patterns during sleep (Shapiro and Hepburn, 1976).

The goal of this research was to determine whether a diel cycle in metabolism exists in cunner and determine its magnitude. The cunner is a good experimental animal because of its tendency to seek cover; therefore it will readily habituate to a small respirometer. It is generally inactive at night and at cold temperatures, therefore maintaining relatively constant metabolic rates during these times.

## MATERIALS AND METHODS

The data for this chapter were collected in the same manner as that described in Chapter 2. In brief, fish were kept in flow-through chambers for several days. The oxygen concentration of the water was determined every 15 minutes. A mean hourly oxygen consumption rate was obtained, and all the consecutive data for each fish were constructed into one time series. Because a one-hour calibration was performed each day, precluding metabolic data from being collected at this time, the oxygen consumption rate for this period was estimated by using the two mean hourly values directly before and directly after the calibration.

The time-series data were collected at ambient water temperature and photoperiod to best reflect natural conditions. The data collected on the first day the fish were placed in the chambers were not used because of the known effect that handling has on fish physiology (Saunders, 1963). This was a conservative precaution; other studies have shown that oxygen consumption rates stabilized after a few hours of handling (Smith, 1978; Smith and Laver, 1981; Smith and Brown, 1983; Du Preez et al., 1986). The decrease in oxygen used on the first day of an experiment can be seen in Figure 5.1. The amount of oxygen actually used by the fish is represented on the y-axis. As the fish were of different weight, the metabolic rates were different.

The data were used to generate a periodogram. The purpose of the periodogram is to partition the variability of the series into discrete frequency components. Because periodograms are not consistent in estimating the power or spectral density function, it is better to estimate the power spectrum after smoothing the periodogram. Therefore, a

smoothed periodogram (i.e. spectral estimate) was generated following the methodology described in Chatfield (1989). It was constructed by smoothing the periodogram using a smoothing neighborhood equal to 15% of the Fourier frequencies. This is a bandwidth that has gained acceptance in the literature (Solow, 1992). The smoothing was performed for the whole series and then a plot of power vs frequency is generated. By noting the frequency at which the power is at a peak, and then converting frequency into time [period =  $(2 \times \pi)/\text{frequency}$ ], one can conclude that the maximum amount of the variability in the time series is attributable to that frequency.

## RESULTS

Representative time series obtained for two different fish used at warm temperatures (Figures 5.2 and 5.3) and time series obtained for two fish used at cold temperatures (Figures 5.4 and 5.5) are presented. It appears that there is a cyclical pattern, but the length of the cycle can best be determined by generating the periodogram. Note that the scale on the y-axes are different. A total of 23 smoothed periodograms were generated, representing different times throughout the year. Depending on the frequency at which the power was at a peak, they were placed into two categories: 1) diurnal cycle or 2) cycle of approximately 48 h (Table 5.1). The data collected at higher temperatures were general diurnal, and those at colder temperatures were approximately 48 hours. The period lengths considered as a 24 h cycle generally ranged from 17.2-29.6 and the period lengths considered as a 48 h cycle generally ranged from 31.3-49.1, although three cycles were approximately 69 h.



To determine whether the amount of variability in oxygen consumption rate differed with season, the standard deviation for each of the time series was obtained and is also presented in Table 5.1. The standard deviation (SD) increased with temperature indicating that the variability of the time series is affected by temperature, being greater at warmer temperatures. The change in mean metabolic rate with temperature was addressed in detail in Chapter 3.

Each periodogram did not have the identical peak frequency because of variability in the data, and because as the length of the time series increases so does the number of Fourier frequencies. Therefore, because each time series is of a different length, the Fourier frequencies are not exactly the same. The corresponding smoothed periodograms for each time series are presented in Figures 5.2-5.5. For the representative time series at warm temperatures (Figs. 5.2 and 5.3), the peak frequency occurs at approximately 0.26, corresponding to a cycle of 24 h. For the representative time series at cold temperatures (2.5°C), the peak frequency occurs near 0.11 approximating a 48 h cycle (Figs. 5.4 and 5.5). The smaller peak that occurs near the frequency of 0.22 indicates that a 24 h cycle may be superimposed on the 48 h cycle, however, the 48 h cycle is predominant. A signed-ranked test was used to determine whether there was a significant difference between day and night rates; the difference was significant at the 0.05 level.

Heart rate was also used as an indicator of metabolism. Cunner were implanted with two electrodes in/near the pericardial cavity, and were monitored for several days. The mean daily and nightly rates are listed in Table 5.2. The heart rate during the day was significantly different than that at night ( $p < 0.05$ ) using a paired t-test.

The difficulty in using heart rate as an indicator of metabolism is the variability that can occur in stroke volume. Priede and Tytler (1977) noted that in fishes the range in variation in oxygen consumption rate for a given heart rate is too wide for any significant correlation to be made. Therefore, heart rate is not as good a measure of metabolism in fishes as it is in other organisms. In some studies, heart rate has proved useful in setting maximum values on metabolism (Priede, 1983), or as an indicator of food intake (Armstrong, 1986). Because limited heart rate data were collected on cunner and longer time series of oxygen consumption rates were collected, no metabolic rate estimates were based on the heart rate data.

## DISCUSSION

Because cunner have marked behavioral changes throughout the diel cycle, it was expected that a periodicity of 24 h would be found in the data. There was a cycle of about 24 h during periods of warm temperature, but in colder temperatures the cycle was about 48 h. Apparently, cunner do not have a consistent year-round diel cycle. The increase in cycle length during the colder months indicates that during the inactive period, when the animal is physiologically torpid, the rhythm is prolonged. Perhaps the 24-h cycle is not of importance during this period; cunner are inactive both day and night and any exogenous cue such as light may no longer stimulates activity. Physiologically, this is reasonable because there is no need for the fish to have a daily increase in metabolic rate when one of the primary functions of hibernation is energy savings.

Although a diurnal rhythm was observed in fish at high water temperatures, there is no evidence to suggest that the rhythm is circadian. To do that, one would have to be assured that the cycle is endogenous [see Pittenridge (1960) for other criteria]. Circadian rhythms, however, are thought to be temperature independent, with  $Q_{10}$  values approximating 1.0 (Pittenridge, 1960). As the diurnal cycle is not maintained throughout the year, it is possible that temperature, or its effect on hibernation, may be influencing the cycle. Therefore, the rhythm may not be endogenous, but be affected by exogenous cues.

In comparing the warm water to cold water spectral estimates (Fig. 5.4 and 5.5), several features stand out. The warm water plot has a peak that is shifted to the right, therefore corresponding to a shorter period than the cold spectral estimates (24 vs 48 h). Also the peak in January is much sharper and the plot is generally less variable. Given that the fish are much less active in cold weather and are rarely seen to move, this result is not unexpected. The decrease in the variability in metabolic rate with decreasing temperature can also be noted in Table 5.1. Therefore, decreasing temperature affects both metabolic rate (Chapter 3), and the variability of these data indicating a decrease in spontaneous activity. An increase in spontaneous activity with increased temperature was also seen in the brown bullhead *Ictalurus nebulosus* (Crawshaw, 1984). The metabolic rate of cunner is statistically more variable in warmer than colder temperatures (Table 5.1).

The heart rate of cunner was also significantly lower at night than during the day. This method, however, is not as accurate as oxygen consumption rates in determining

metabolic rate because stroke volume can vary, but this preliminary study was performed before the oxygen electrode system was available. As the fish were constrained within a small area, stroke volume changes related to vigorous exercise were minimized. Belich (1984) determined that heart rate could be used to define the wake-rest cycle in the catfish *Ictalurus nebulosus*. The heart rate data on cunner and the data on the oxygen consumption rates conclude that cunner have a strong rhythm in metabolic rate.

Table 5.1  
 Cycle Length of Cunner at Different Temperatures and Months  
 With Standard Deviations of the Oxygen Consumption Rates

Diurnal			48 Hours		
TEMP (°C)	MONTH	SD	TEMP (°C)	MONTH	SD
22.9	August 1990	9.1	4.3	January 1992	2.4
22.9	August 1990	7.0	2.5	January 1992	3.9
22.9	August 1990	3.9	2.5	January 1992	4.1
7.8	December 1990	2.6	2.5	January 1992	3.5
6.9	January 1991	2.4	3.6	February 1992	1.8
16.6	October 1991	4.4	3.6	February 1992	2.5
16.6	October 1991	5.4	8.8	April 1992	3.2
16.6	October 1991	4.2	8.8	April 1992	3.3
7.8	December 1991	2	8.8	April 1992	1.6
7.8	December 1991	2.2			
4.3	January 1992	2.3			
4.3	January 1992	1.7			

Table 5.1

Individuals were placed in one of two categories (Diurnal or 48 hours) based on the length of their oxygen consumption rate cycle as determined from the smoothed periodogram. All of the warm temperature time series had a diurnal cycle. The cold temperature cycles approximated 48 h, with the exception of two fish observed at 4.3°C. This temperature may be near the point at which fish enter hibernation. The variability of the oxygen consumption rate, as measured by standard deviation (SD) was higher during the warmer temperatures than the lower temperatures. This indicates that spontaneous activity was more variable at higher temperatures. Mean oxygen consumption rates for 54 fish are presented in Table 3.1.

Table 5.2  
Mean Heart Rate (Beats Per Minute) of Cunner

SAMPLE	CUNNER 1		CUNNER 2		CUNNER 3		CUNNER 4	
	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
1		87		64	66		43	39
2	78	77	95	83	56		52	
3	100	86	72	52		58		
4	102		80	52	51			
5	105		73					

Table 5.2

Cunner heart rate was monitored for several days using electrodes placed near the pericardial cavity. The heart rate of cunner was higher during the day than at night as determined by a paired t-test ( $p < 0.05$ ).

Figure 5.1

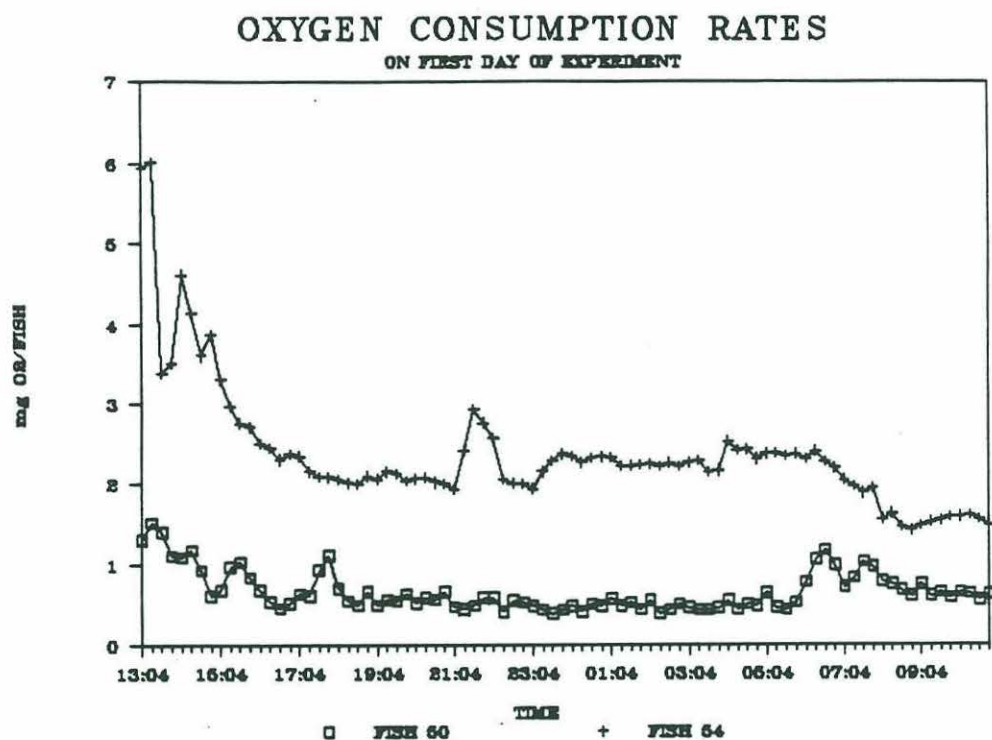


Figure 5.1

The oxygen consumption rates of cunner were much higher on the day they were placed in the respirometry chamber and were not used in the analysis. Data on two fish were presented to show this trend. Note that the metabolic rate becomes more constant after approximately 4 h.

Figure 5.2

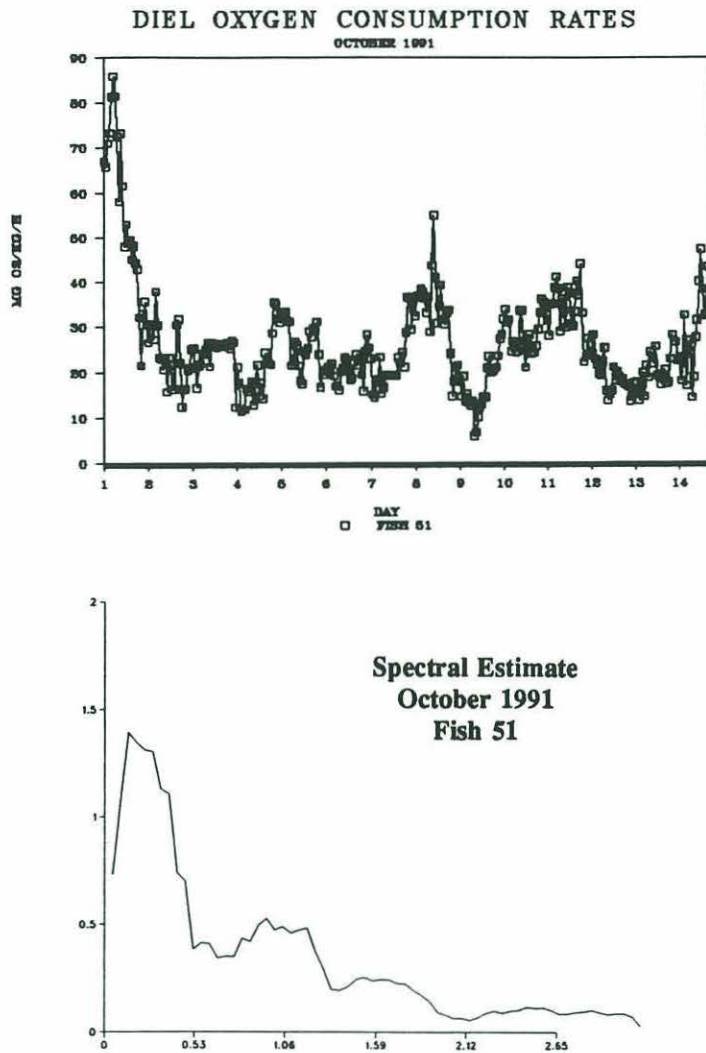


Figure 5.2

*top* The change in oxygen consumption rate in one individual over the course of a 13-d experiment in October is presented. Time along the x-axis is denoted by approximately noon (12:06) each day. As expected the lower oxygen consumption rates usually occurred at night.

*bottom* The corresponding spectral estimate, or smoothed periodogram, is a plot of power vs frequency and is used to describe the amount of variability in the data that is attributable to various frequencies. The frequency (x-axis) at which power (y-axis) is greatest corresponds to a period of approximately 24 hours indicating that cunner are diurnal at warm temperatures.



Figure 5.3

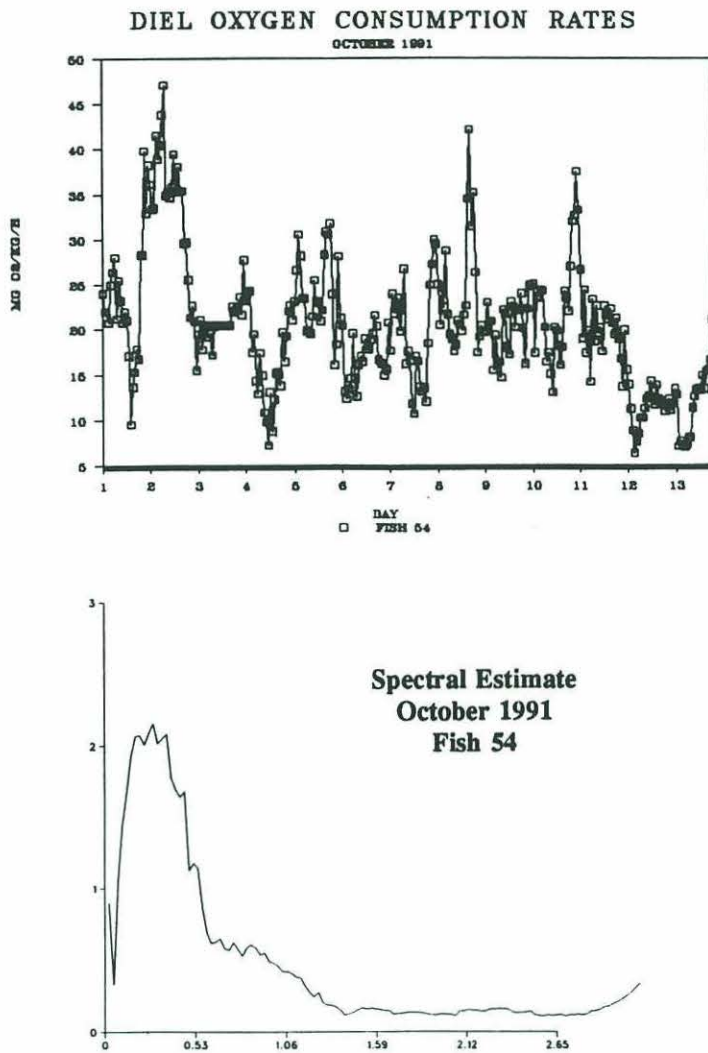


Figure 5.3

*top* The change in oxygen consumption rate in one individual over the course of a 13-d experiment in October is presented. Time along the x-axis is denoted by approximately noon (12:06) each day. As expected the lower oxygen consumption rates usually occurred at night.

*bottom* The corresponding spectral estimate, or smoothed periodogram, is a plot of power vs frequency and is used to describe the amount of variability in the data that is attributable to various frequencies. The frequency (x-axis) at which power (y-axis) is greatest corresponds to a period of approximately 24 hours indicating that cunner are diurnal at warm temperatures.

Figure 5.4

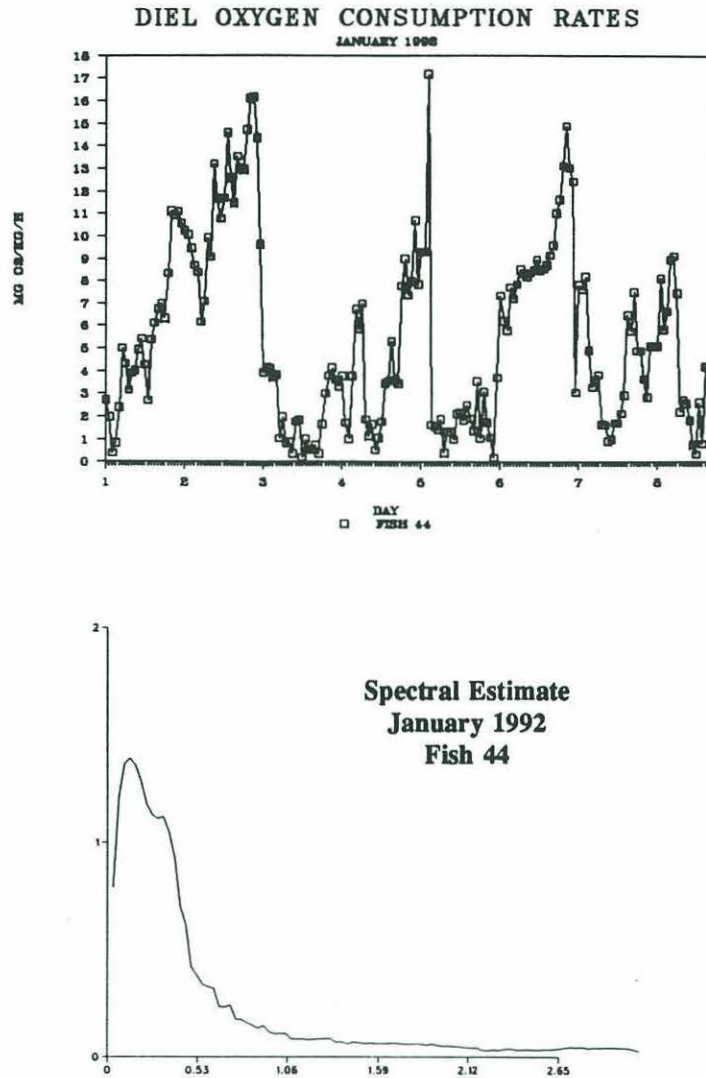


Figure 5.4

*top* The change in oxygen consumption rate in one individual over the course of a 13-d experiment in January is presented. Time along the x-axis is denoted by approximately noon (12:06) each day. As expected the lower oxygen consumption rates usually occurred at night.

*bottom* The corresponding spectral estimate, or smoothed periodogram, is a plot of power vs frequency and is used to describe the amount of variability in the data that is attributable to various frequencies. The frequency (x-axis) at which power (y-axis) is greatest corresponds to a period of approximately 48 hours, demonstrating that the cycle in cunner is no longer diurnal.

Figure 5.5

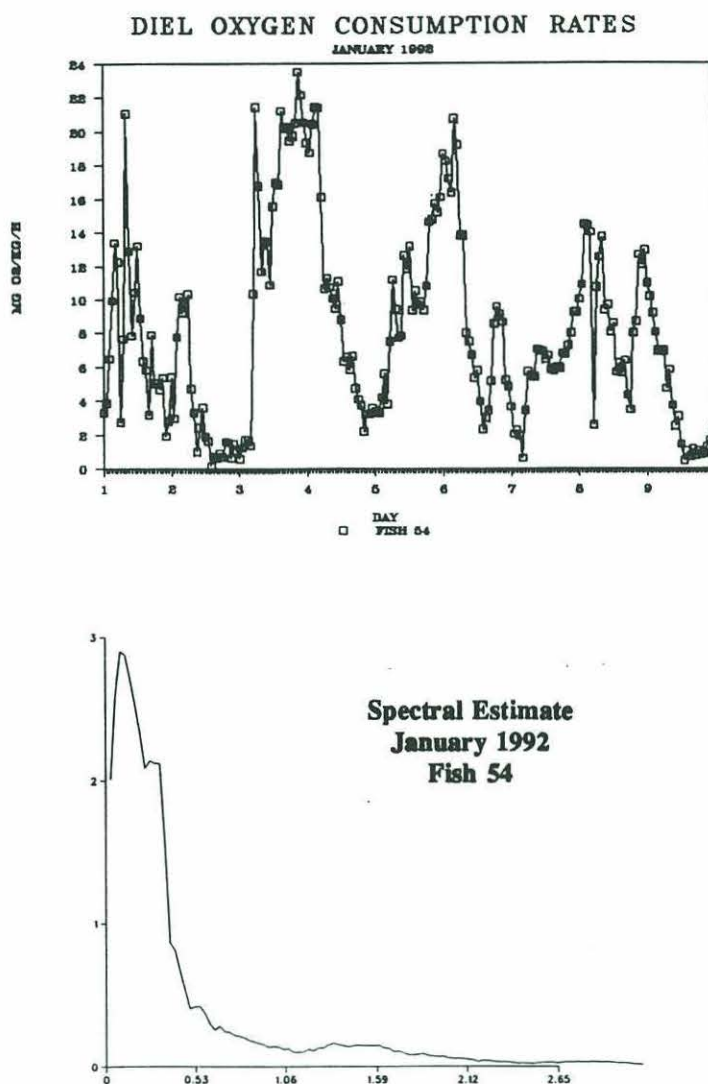


Figure 5.5

*top* The change in oxygen consumption rate in one individual over the course of a 13-d experiment in January is presented. Time along the x-axis is denoted by approximately noon (12:06) each day. As expected the lower oxygen consumption rates usually occurred at night.

*bottom* The corresponding spectral estimate, or smoothed periodogram, is a plot of power vs frequency and is used to describe the amount of variability in the data that is attributable to various frequencies. The frequency (x-axis) at which power (y-axis) is greatest corresponds to a period of approximately 48 hours, demonstrating that the cycle in cunner is no longer diurnal.



## CHAPTER 6

### DIEL CHANGES IN OXYGEN CONSUMPTION RATES IN TROPICAL LABROID FISHES

#### INTRODUCTION

Tropical members of the family Labridae (wrasses) and the closely related family Scaridae (parrotfishes) exhibit three distinct behavioral/physiological traits that are unique to this group, which make them ideal for studying diel changes in behavior and physiology. Some species:

- 1) have settlement-stage larvae that bury themselves in the sand and emerge as juveniles several days later (Victor, 1983);
- 2) are specialized for seeking refuge under sand. These species typically bury themselves each night to sleep, and can dive into the sediment at any time when threatened (Hobson, 1968; 1974); or
- 3) secrete a mucous cocoon at night (Byrne, 1970).

Wrasses and parrotfishes are diurnal, feeding during the day and resting at night (Hobson, 1972). Only some species are capable of burying themselves (Table 2.1), or constructing a mucous cocoon (Table 2.2). The cocoon is a thin, clear mucous bubble that encases the fish, except for a flap-covered whole over the mouth, and a small opening at the rear to rid respiratory water (Winn, 1955). It is usually secreted at night, but is also secreted in the day during periods of low oxygen availability. Cocooning may protect individuals from predators because it contains its scent. Therefore, a cocooning fish may be able to reduce metabolism at night more than a non-cocooner because it would be less likely to

have to flee from a predator. Further evidence suggesting that fishes reduce their metabolism during cocooning is that the only other time cocooning behavior occurs is during periods of low oxygen availability. There has also been no comprehensive study of the changes in metabolic rates that occur. The cocooning ability may be a "key innovation," and the ability to reduce metabolic rates when stressed possibly enabled the labroids to inhabit new areas. The aim of this study was to determine the amount of metabolic reduction that occurred during periods of rest at night.

## MATERIALS AND METHODS

Fishes were collected at the Discovery Bay Marine Laboratory in Discovery Bay, Jamaica, using a barrier net. After being measured and weighed, fishes were placed in flow-through chambers (as described in Chapter 3) and oxygen consumption rates were monitored for three consecutive days. The species used were the striped parrotfish *Scarus iserti*, the bluehead wrasse *Thalassoma bifasciatum*, the damselfish *Eupomacentrus leucostictus* and a non-labroid, the nocturnal squirrelfish *Holocentrus rufus*.

Because of the limited time spent in Jamaica, fishes were placed in respiratory chambers upon hours of capture and were only observed for three days before new individuals were used. The recurring problem of power outages precluded continuous recordings of respiration rate. Therefore, results for this chapter are presented as a mean of the daytime rates (0600-1800) and compared to those at night (1800-0500).

Prior to obtaining the oxygen electrode system, changes in metabolic rate were established by counting opercular flap movements. Observations per fish were made for

20 sec and three replicates were taken within an observation period of about 10 min. Each fish was observed every 2 h and maintained in the laboratory at least 2 d.

## RESULTS

The mean oxygen consumption rate values during the day and night for ten individuals are presented in Figure 6.1. The error bars are  $\pm 1$  SE and the sample size (n) represents the number of day/night observations. The night values for *S. iserti* and *Thalassoma bifasciatum* were significantly lower than the day values. *Eupomacentrus leucosticus* had values that were nearly the same both day and night. *Holocentrus rufus* had higher values at night than during the day.

The mean value for each species is presented in Figure 6.2. As only one individual of *Eupomacentrus leucosticus* and *Holocentrus rufus* were used, there are no SE bars for these species. The sample size (n) indicates the number of individuals used. The daytime rates in *Scarus iserti* and *Thalassoma bifasciatum* were significantly higher than those at night using a paired t-test.

The diel changes in opercular beats per minute is found in Fig. 6.3. The parrotfishes (first three species) had a marked day/night difference. the wrasses (second three species) did not have as large a difference as the scarids. The damselfish (pomacentrid) had a higher metabolic rate during the day. Although not significant, the nocturnal squirrelfish *Holocentrus rufus* had a nocturnal rate that was higher than the rate during the day, as did the other nocturnal species *Myrichthyes oculatus* and *Atherinimorus stipes*.

## DISCUSSION

A marked change in diel oxygen consumption rate occurred in the parrotfish, wrasse and squirrelfish (Fig. 6.2). This is not surprising given their activity patterns. The damselfish, although it rests at night, still maintains itself in the water column and uses its fins. Its rates during the day and night were similar. The squirrelfish had a lower rate at night than during the day, which was expected because it is nocturnal. Unlike the temperate experiments, time limitations precluded fishes from becoming acclimated to the laboratory before use, nor was the prior feeding history known. Both these factors may cause the data to be more variable and possibly lead to artificially high results being recorded.

Metabolic rate can be estimated by counting opercular flap movements. This method cannot be used to evaluate actual amounts of oxygen consumed, but may be useful in determining relative oxygen requirements.

Similar results were obtained using this technique. The labroids had higher rates during the day, and nocturnal species had higher rates at night. Byrne (1970) obtained opercular counts of cocooning scarids. He determined that respiration rates decreased markedly at night in resting scarids that formed cocoons. Opercular flap movement in both *Scarus dubius* and *S. perspicittatus* decreased 46% (n=3) after the cocoon was constructed. *Scarus iserti* had rates during the day between 73-144 beats per minute (BPM) and rates at night between 47-56 BPM (Winn, 1955). In *Halichoeres bivittatus* nocturnal rates were approximately 30 BPM, while during wakefulness the values ranged



from 70-140 BPM (Tauber and Weitzman, 1969). Daytime values were 60-70 BPM in *Sparisoma aurofrenatum*, while nocturnal values ranged from 24-60 BPM.

The data on opercular beats per minute showed similar trends. *Scarus iserti*, *Sparisoma aurofrenatum*, and *S. radians* had significantly different values during the day than at night. Three wrasse species did not show significant differences (*Halichoeres garnoti*, *H. bivittatus*, and *Thalassoma bifasciatum*).

The inactivity of labroid fishes at night and their ability to sleep has been well documented. Labrids are one of the first species to take cover at night, and the smallest species generally take cover first. Within a species, the larger individuals are often the last to retire at night and the earliest to emerge in the day (Hobson, 1972). Many small individuals within a species are more likely to form cocoons (Hobson and Chave, 1972), and its construction may increase predator protection (Hobson, 1965). As weight-specific metabolic rate is higher in smaller individuals, smaller individuals are better off spending as much time as possible in an inactive state. Several scarid species secrete cocoons during the day under anoxic conditions (Winn, 1955; pers. obs.), indicating that its construction may be induced by stress and may ultimately provide some metabolic savings.

Sleep is thought to be physiologically advantageous because of the energy savings associated with reduced activity, and in the case of mammals and terrestrial ectotherms, reduced body temperature. This "voluntary hypothermia" is thought to reduce energetic costs and is maximized by the selection of optimal sleeping sites in the land iguana *Conolophus pallidus* (Christian and Tracy, 1984).

Birds and most mammals have two sleep phases: Slow Wave Sleep (SWS) that has EEG activity with high voltage slow waves, and Paradoxical Sleep (PS) or Rapid Eye Movement (REM), that follows SWS. The percentage of sleep that is occupied by PS varies from species to species, but is generally lower in birds than in mammals (Ayala-Guerrero, 1989). In mammals, the time spent in quiet sleep was negatively correlated with body size and basal metabolic rate (Elgar et al., 1988).

In an ectotherm such as a tortoise, there were also two forms of sleep: quiet sleep during which the brain exhibited little activity, and active sleep during which the brain resumed fast activity as if the animal were awake (Ayala-Guerrero et al., 1988). The animals demonstrated swimming behavior in active sleep, moved their heads, and exhibited REM as in mammalian sleep. But only 2% of their sleeping time was spent in this mode. Heart rate and respiratory rates were lower during slow wave sleep than wakefulness, but these rates increased slightly during paradoxical sleep (Ayala-Guerrero et al., 1988). As previously mentioned, several labrids have characteristics similar to mammalian sleep. There is overall inactivity, a decrease in response to stimuli, a diminished and irregular respiratory rate, and independent eye movement (Tauber and Weitzman, 1969). In the laboratory, resting labrids can be brought to the surface before becoming fully alert (Tauber and Weitzman, 1969). No REM was found in the cichlid *Tilapia mossambica* (Shapiro and Hepburn, 1976), but there was evidence of behavioral sleep in terms of a reduced response to stimuli (feeding and electric shock), and a stereotypic sleeping posture. These fish did not rest on the bottom during continuous illumination. Characters other than behavioral cues can be used to determine if fish are

sleeping. Belich (1984) determined that heart rate could be used to define the wake-rest cycle in fish.

The data presented suggest that there is a decrease in metabolic rate in the labroid fishes. Previous research has demonstrated that labroids sleep. Additionally, some species secrete a cocoon during periods when oxygen levels are reduced. Both these features collectively suggest that labroids are well-adapted for energy conservation.

Figure 6.1

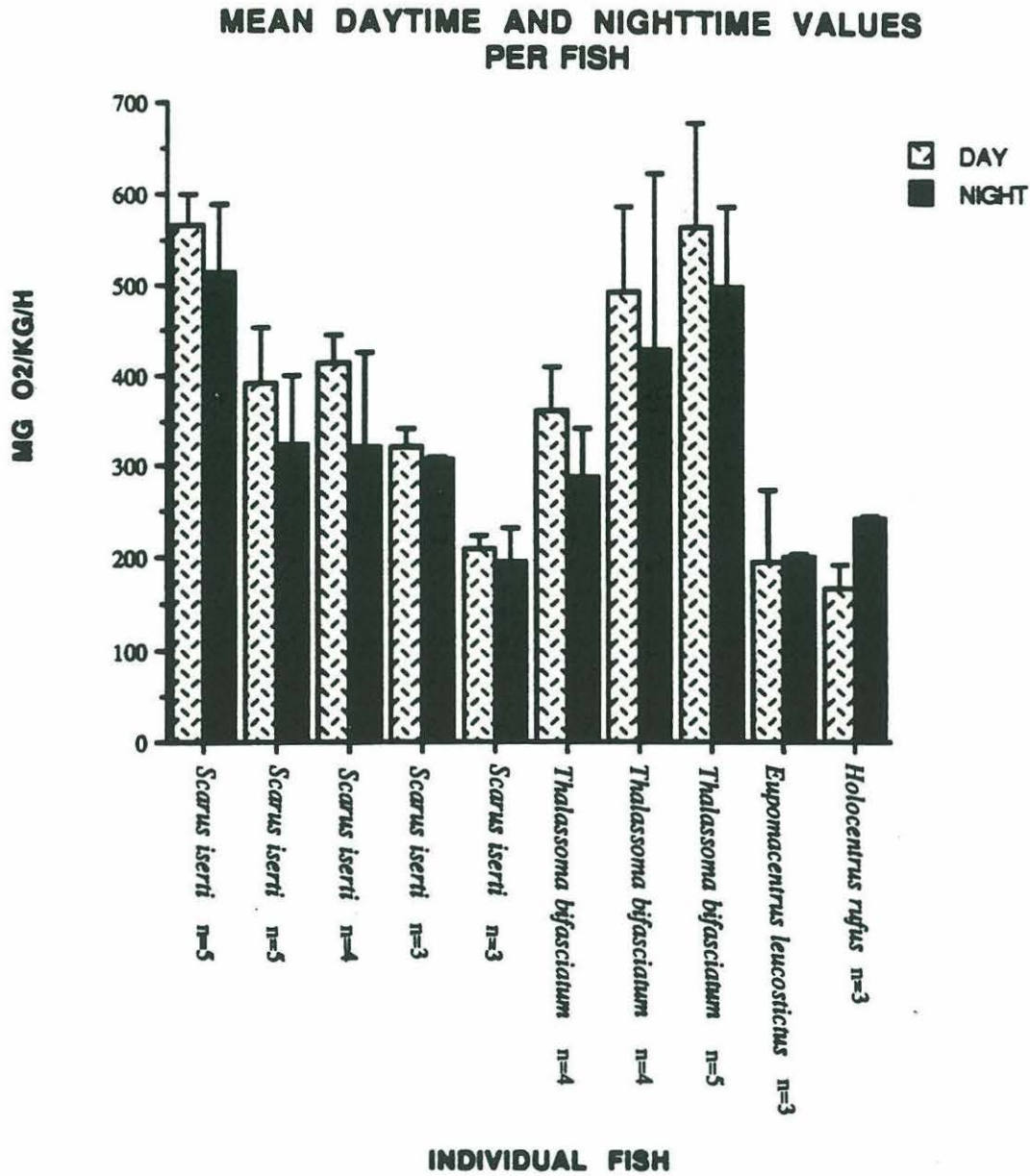


Figure 6.1

The mean oxygen consumption rates ( $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) during the day and night for ten individuals is presented. The labroids (*Scarus iserti* and *Thalassoma bifasciatum*) had higher values during the day than at night. The damselfish *Eupomacentrus leucostictus*, a species that is not as inactive at night as the labroids, had nearly identical rates during day and night. The nocturnal squirrelfish *Holocentrus rufus* had a rate that was higher at night than during the day. Sample size (n) represents the number of days that each fish was used. Error bars are  $\pm 1$  SE.

Figure 6.2

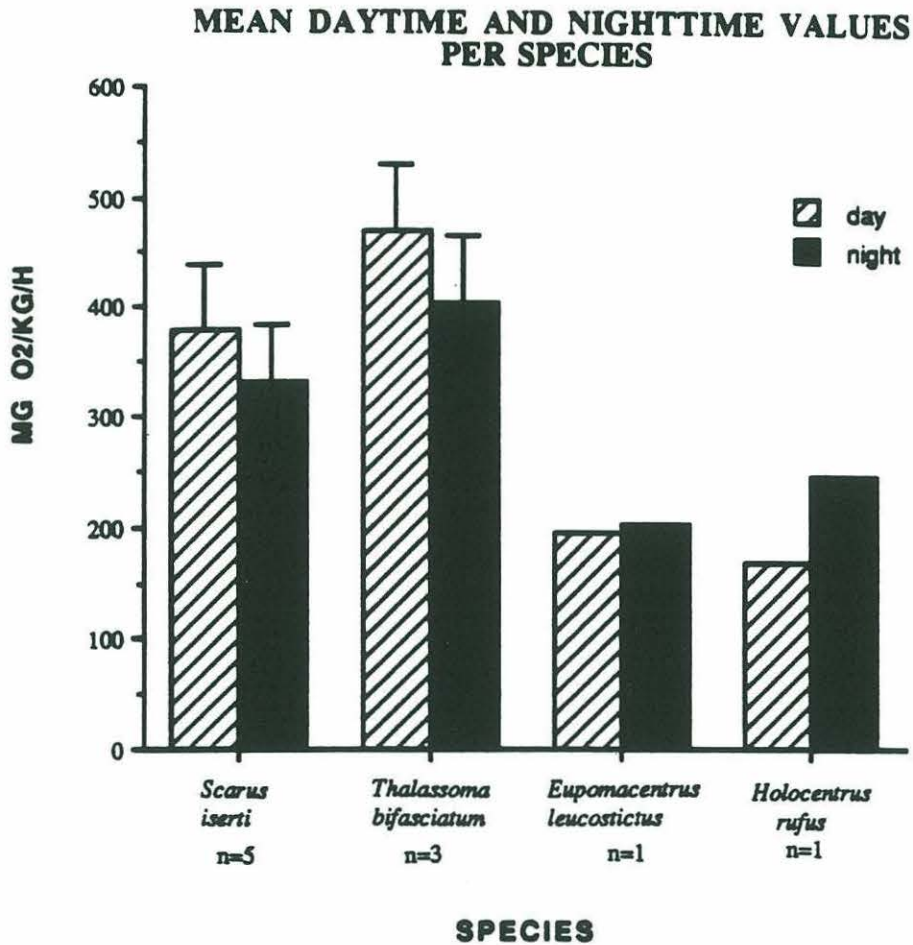


Figure 6.2

Mean oxygen consumption rates ( $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) during the day and night are presented for each species. For the labroids *Scarus iserti* and *Thalassoma bifasciatum* the rates during the day were significantly higher than those at night as determined by a paired t-test ( $p < 0.05$ ). No statistics were performed on *Holocentrus rufus* and *Eupomacentrus leucostictus* as only one individual was used, although it appears that the nocturnal squirrelfish *Holocentrus rufus* has a rate that is greater at night than during the day, and that the damselfish *Eupomacentrus leucostictus* did not have a diel difference. Sample size (n) denotes the number of individuals used. Error bars are  $\pm 1$  SE.

Figure 6.3

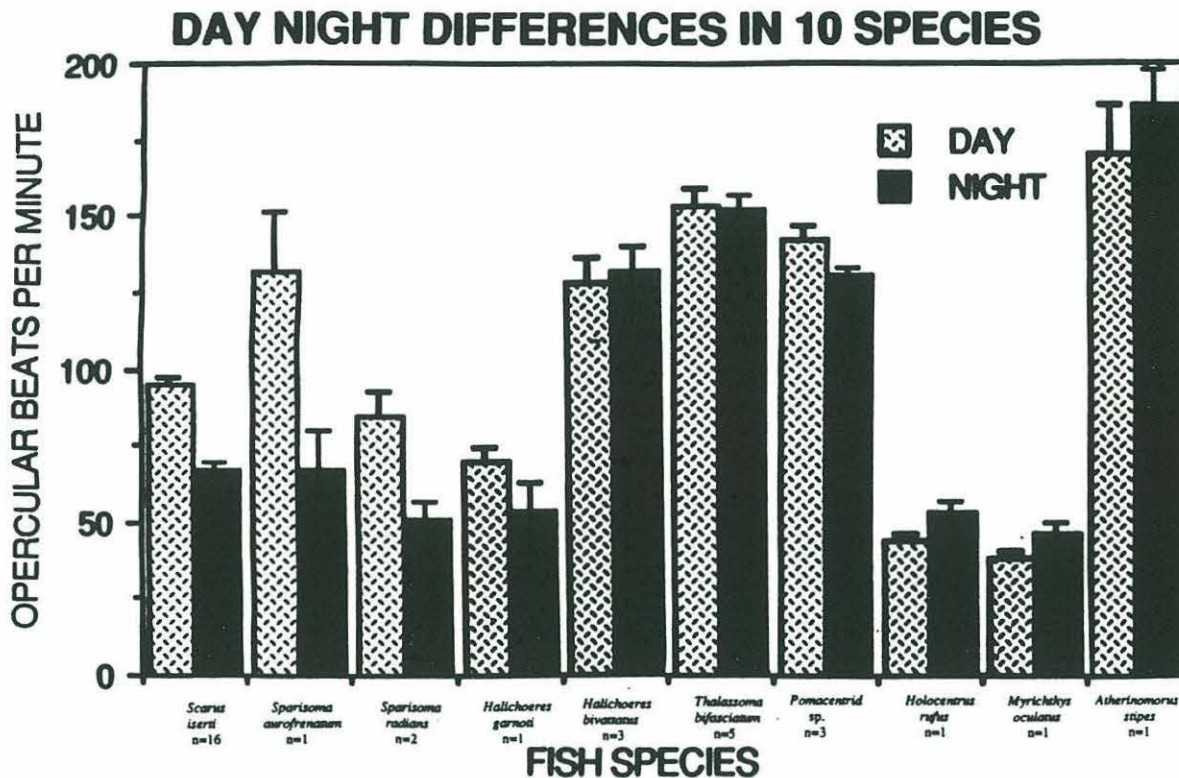


Figure 6.3

Metabolic rate was estimated by counting the number of opercular beats per minute. The scarids (first three species) had a markedly higher day rate than night rate, although only *S. iserti* had a significant difference using a paired t-test ( $p < 0.05$ ). The wrasses (*Halichoeres* spp. and *Thalassoma bifasciatum*) were inconsistent with respect to a diel pattern. Although not significant, the nocturnal squirrelfish *Holocentrus rufus* had a nocturnal rate that was higher than the rate during the day, as did the other nocturnal species *Myrichthys ocellatus* and *Atherinomorua stipes*.

## CHAPTER 7

### SUMMARY AND PERSPECTIVES

This dissertation focuses on behavioral and physiological aspects of the activity and metabolism of labroid fishes in temperate and tropical waters. This group, which is of tropical origin, exhibits several unusual behaviors. Some tropical species can construct a mucous cocoon, either at night as a defense against predation, or during the day under conditions of low oxygen tension. Some species can bury themselves in the sediment. Like cocooning, this usually occurs at night and may serve as an anti-predatory device, although individuals can remain buried for days. During both of these behaviors, reduced oxygen availability may require a reduction in metabolic suppression in labroid fishes.

The two temperate species in the Western North Atlantic do not cocoon, although they are inactive at night. Based on behavioral observations, both the cunner *Tautoglabrus adspersus* and the tautog *Tautoga onitis* appear torpid. They are two of the few fish species of that remain inshore during winter when other species move offshore into warmer waters. The ability of labroids to reduce their metabolism more than other species may have predisposed them to survive these extended periods of inactivity without food.

This work is unique in addressing both diel and seasonal changes in metabolism in related tropical and temperate species by determining oxygen consumption rates continually over long periods. It provides insight into controlled metabolic reduction in animals and potentially redefines baseline measurements in ectotherms. This study also combines the fields of both behavior and physiological ecology as it addresses the

concomitant changes in physiology that accompany periods of inactive behavior, and the possible evolutionary role of this metabolic change in the biogeographic distribution of modern-day fishes.

The  $Q_{10}$  value, calculated from oxygen consumption rates over a wide range of temperatures, indicates that cunner hibernate at temperatures below 6.4°C. Below 6.4°C, metabolic rate was relatively independent of temperature, whereas the  $Q_{10}$  value between 12 and 22°C was 2.4. A  $Q_{10}$  of 8.5 for the temperature range between 6.4 and 12°C clearly shows that metabolic rate in this species changes more than expected on the basis of temperature alone. The large  $Q_{10}$  value of 8.5, calculated using oxygen consumption rates, provides evidence that cunner hibernate. Other investigations have documented hibernation in ectotherms including turtles, snakes, and freshwater fishes; these organisms may also experience anoxic conditions during hibernation. Few marine fishes have previously been shown to hibernate, although the eel *Anguilla anguilla* was found to have a  $Q_{10}$  value above 4 (Walsh et al., 1983), suggesting that it may also hibernate.

Starvation during hibernation in cunner reduced liver concentrations of glycogen, lipid, and protein, while body stores remained largely unchanged. Liver glycogen reserves comprised a major portion of both liver weight and energy reserve, suggesting that earlier studies may underestimate the importance of this storage product in fishes. The rate of decrease of liver reserves during hibernation implies that cunner could potentially live six months on their glycogen and lipid reserves, and nine months on protein stores, although some lipid and protein are not available for energy as they are required for structural integrity. Although the concentration of energy reserves is lower



in muscle, the muscle comprises a large percent of the mass of the fish; cunner could probably survive many additional months based on these reserves. Based on the depletion of energy reserves observed, cunner could live well over a year during periods of food deprivation at 4°C. Therefore, the northern biogeographical limit of cunner is probably not restricted by their ability to survive long periods of cold-water starvation during hibernation.

The northern biogeographical limit of cunner is near Logy Bay, Newfoundland (J.M. Green, pers. comm.). The mean monthly water temperatures in this area (Steele, 1974) are presented in Table 7.1 along with the mean monthly water temperatures in Woods Hole, MA. In Woods Hole, cunner encounter three months during which the water temperature is below 4°C, whereas in Newfoundland, water temperatures remain below 4°C for six months. Cunner can remain behaviorally torpid during this time (J.M. Green, pers. comm.). That they can survive six months without eating is consistent with the findings of this dissertation that energy reserves can last at least six months at 4°C. Furthermore, Green (pers. comm.) noted that the northern distributional limit of cunner is not marked by a sharp thermal gradient, nor is there a dispersal barrier at this point. Instead, the northern limit may be determined by the amount of time available for eggs and young to develop before the onset of temperatures at which they become torpid. Therefore any global increase or decrease in temperature would have to affect temperatures for periods on the order of weeks or months for the northern limit of cunner to be affected.

A number of other observations suggest that cunner are well-adapted to permanent inshore life. Peripheral tissues, such as the skin, gills, and corneal epithelia, exhibit thermal hysteresis activity (a gap between the melting and freezing points) indicating the presence of antifreeze polypeptides (Valerio et al., 1990). Contact with ice-laden water is reduced by huddling in groups under rocks (Valerio et al., 1990; Curran, pers. obs.). The production of copious amounts of mucus at low temperatures may also be a strategy to reduce skin contact with ice crystals (Valerio et al., 1990; Curran, pers. obs.). As oxygen demands decrease during inactivity at cold temperatures, so does gill ventilation, further reducing exposure to water containing ice crystals (Valerio et al., 1990). The development of this adaptation in cunner must be relatively recent evolutionarily since labrids are tropical in origin (Valerio et al., 1990). Labrids therefore exhibit two characteristics that enable them to survive inshore in temperate areas throughout the year: the ability to hibernate, and the thermal hysteresis activity exhibited in peripheral tissues. These two adaptations are significant in light of the tropical origin of this family, providing an example of how tolerance to the physical environment (temperature) allows habitat exploitation; species that cannot tolerate low temperatures must leave (Tracy and Christian, 1986). The case of cunner illustrates the need to consider temporal scales when establishing species' home ranges. The capability of cunner to be more eurythermic than species that must leave indicates that they may have enzymes more tolerant to temperature, enabling the exploitation of a habitat unoccupied by other species (Tracy and Christian, 1986).

The inactivity of cunner, or at least the lack of vigorous activity at low temperatures, may be partly caused by the failure of coordinated muscle activity below the normal threshold of exhaustion due to temperature-sensitive enzymes (Dalla Via et al., 1989). Also, upon exercise, the return of the metabolic byproducts to pre-exercise levels takes 4 x as long at 4°C than at 20°C in *Rutilus rutilus*. This further illustrates the disadvantage of activity at low temperature.

Tropical fish species, including labroids, enter temperate waters during the late summer and early fall, but are thought to die as seasonal water temperatures become colder. This is also a case where dispersal factors are not restricting the northern limit of tropical species. Instead, temperature tolerance may be limiting.

The diurnal cycle in cunner metabolism was not maintained at colder temperatures. The cycle extended to approximately 48 hours during the period in which cunner were hibernating. The biological significance of this finding is difficult to explain. It is possible that this is another energy-conserving mechanism; it is optimal for cunner to remain as inactive as possible during winter. Since they are not feeding during this time, a relationship between daylight and feeding habits need not be maintained.

Tropical members of the same family (Labridae) and the closely related parrotfishes (Scaridae) exhibited significant differences in diel oxygen consumption rates; the metabolic rates were higher in the day than at night. Perhaps the predisposition of temperate labroids to reduce metabolism both at night and during hibernation has enabled them to survive in the Western North Atlantic.

There are many directions this work could be pursued in the future. Firstly, one of the most interesting findings was the change in cycle length from 24 to 48 hours as temperature decreased. No information was gathered to elucidate whether or not the underlying mechanism for the rhythmicity was endogenous or exogenous. The fact that the period of the cycle changes with temperature suggests that the cycle is not endogenous; circadian rhythms are temperature independent. However, the possible effects of light need to be examined. If the observed rhythms were maintained when fish are kept in constant light or constant dark, this would constitute evidence that the cycle is endogenous. Performing these experiments over a range of temperatures would clarify the possible role of light throughout the seasonal cycle. Quantification of seasonal changes in activity would be useful to determine metabolic change attributable to temperature versus activity.

Evidence for hibernation in cunner is based on oxygen consumption rates collected over a range of temperatures and the subsequent calculation of  $Q_{10}$  values. Other proof of internal activity changes could be obtained. Evidence of changes in activities and concentrations of enzymes would be particularly interesting given that starvation during hibernation may affect the way in which energy stores are used. Enzyme activity levels have been examined in European eels *Anguilla anguilla* that were starved at 5°C and those that were fed at 15°C (Walsh et al., 1983). Most enzyme activities displayed no change. Only four enzymes had a decrease in activity: liver 6PGDH, and red muscle PFK, PK, and 3CoADH (Walsh et al., 1983). These enzymes would be the likely choices on which to begin a biochemical study of hibernation in cunner. Finally, a study of acid-

base regulation of enzyme activity might provide insight into mechanisms controlling decreases in metabolic rate during hibernation. All of these topics would be fruitful areas of further research.

Table 7.1

Mean Monthly Water Temperatures:  
Woods Hole vs Logy Bay, Newfoundland

MONTH	WOODS HOLE TEMP (°C)	LOGY BAY TEMP (°C)
JANUARY	2.6	0.1
FEBRUARY	1.2	-0.2
MARCH	2.9	-1.0
APRIL	6.3	-0.1
MAY	13.8	1.9
JUNE	18.7	5.3
JULY	21.6	8.5
AUGUST	22.7	11.0
SEPTEMBER	20.3	11.5
OCTOBER	16.3	8.0
NOVEMBER	10.2	4.8
DECEMBER	6.2	2.8

Table 7.1

The mean monthly water temperatures between Woods Hole and Logy Bay, Newfoundland are compared. Cunner spend about three months at temperatures below 4°C, whereas Newfoundland cunner experience that temperature for about six months. Data from the starvation study (Chapter 4) corroborate that cunner can survive six months based on their energy reserves.

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## BIOGRAPHY

Carla was born in Pittsburgh, PA sometime during the latter half of the 20<sup>th</sup> Century, and spent most of her formative years in the Pocono Mountains of Pennsylvania. She was graduated from Stroudsburg High School in 1979. She then spent a year in Australia as a Rotary Exchange Student where she attended high school for half the year and studied in the marine biology program at James Cook University for the other half of the year. Upon returning to the US, she attended the University of South Carolina from which she was graduated in 1984 with a B.S. in Marine Science. The summer of 1982 was spent at the Harbor Branch Foundation studying seagrass colonization with Dr. Bob Virnstein. The summer of 1984 was spent at WHOI as a Summer Fellow with Dr. J. Frederick Grassle during which a study of Georges Bank sand dollars was completed. In 1984-85 Carla spent a year and a half in New Zealand as a Fulbright Scholar. She received a B.Sc. Honours degree in Zoology after having completed a project on the feeding habits of an echinoderm-eating flounder. In 1986, she began the Ph.D. program at MIT/WHOI with Dr. Phillip Lobel. She has accepted a NSF/NATO postdoctoral fellowship to Naples, Italy where she will be conducting research on sex change in Mediterranean fishes.