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Endocrinological and Environmental Cues Underlying the Seasonal Migrations of Atlantic Cod (*Gadus morhua* L.)

by

Luc A. Comeau

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
Canada

August 2001

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ABSTRACT

Southern Gulf of St. Lawrence cod (*Gadus morhua* L.) perform extended (> 500 km) seasonal migrations, moving from coastal to offshore areas in autumn, overwintering in deep slope waters, and returning to more inshore areas in the spring. However, little is understood about the underlying mechanism controlling these migrations, and the year-to-year variability in the timing of migrations. The objective of this thesis was to identify the physiological and environmental factors implicated in the regulation of cod migration. The approach was based on determining whether the onset of migration is at a fixed level (or phase) within the naturally occurring physiological and environmental cycles. A migration-timing index was computed across several years using fishers' private log records (1970-1992) and sentinel survey data (1995-1999). Between 1994 and 1998, nearly 2,000 cod serum samples were collected and assayed for the determination of various endocrine (gonadal and thyroidal) hormones. Food intake, energy stores, environmental temperature and dissolved oxygen were also closely monitored.

I found little evidence supporting the hypothesis that gonadal hormones (testosterone, estradiol-17ß) are functionally tied to migratory behaviour. On the other hand, I could not reject the hypothesis that thyroid hormones are involved in migration. L-thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) levels were significantly higher at the onset of the autumn migration than during the summer residency period. Moreover, during the return migration in spring, a larger fraction of the metabolically potent T_3 hormone was released from plasma binding proteins, and thus made available for cell uptake. These observations are consistent with the documented role of thyroid hormones in increasing swimming capabilities in the laboratory, and reinforce the view that thyroid hormones facilitate seasonal migrations. With respect to experimental work, this thesis has demonstrated that sustained (1 month) experimental hyper- and hypothyroidism is feasible in wild cod by osmotic pump delivery of thyroid or anti-thyroid agents. Tracking thyroid-manipulated subjects in their natural environment would represent a straightforward approach for verifying the postulated link between the thyroid and migration.

Cod initiated the autumn migration progressively earlier starting in the mid-1980s. At the onset of the autumn migration in the 1990s, dissolved oxygen concentrations were above levels known to induce avoidance behaviour in this species, and food resources were considered to be relatively abundant. Autumn migration onset occurred neither at a specific temperature nor following a particular temperature regime during the previous summer. However, cross correlation function analysis indicated that a widespread cooling of near bottom waters preceded the mid-1980s shift in migration timing by 1 to 2 years, suggesting that the cooling event was linked in some way to the change in migration dates. Other correlations showed that the shift in timing coincided with a reduction in growth rates and also with a rise in the abundance of fish prey inside the southern Gulf. I propose that a reduction in metabolic requirements coupled to an increase in the consumption of fish prey led to a rapid build-up of energy reserves on summer grounds, and to an earlier readiness to engage in the overwintering migration. I conclude by discussing the potential impact of earlier departures on stock productivity.

LIST OF ABBREVIATIONS AND SYMBOLS

km	kilometre
m	metre
cm	centimetre
mm	millimetre
c.	approximately
FL	fork length
I_G	gonadosomatic index
I_H	hepatosomatic index
h	hour
min	minute
°C	degrees Centigrade
°	degree (latitude, longitude)
′	minute (latitude, longitude)
g	gram
mg	milligram
µg	microgram
ng	nanogram
%	percentage
l	litre
ml	millilitre
µl	microlitre
mM	millimole
N	normality
MBq	megaBecquerel
cpm	count per minute
^{125}I	iodine-125
CTD	conductivity-temperature-density (Instrument)

ACKNOWLEGEMENTS

I am particularly indebted to Steven Campana, my supervisor, who has been a valued source of advice for many aspects of my Ph.D., and who has given me a chance to study such a fascinating topic as fish migration. I have enjoyed my time in his lab at the Bedford Institute of Oceanography, and also at sea aboard numerous research and commercial fishery vessels. I am also grateful to my thesis supervisory committee (Timothy Lambert, Hal Whitehead, and Jeffrey Hutchings), and Ghislain Chouinard and Mark Hanson for commenting on specific chapters.

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PUBLICATIONS

I was the principal investigator and author for all publications listed below. The co-authors have been a source of helpful discussions, and commented on draft manuscripts.

Chapter two has been published in:

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Chapter three is in press:

Comeau, L. A., S. E. Campana, G. A. Chouinard, and J. M. Hanson (in press). Timing of Atlantic cod (*Gadus morhua* L.) seasonal migrations in relation to serum levels of gonadal and thyroidal hormones. Marine Ecology Progress Series 00: 00-00.

Chapter four has been submitted for publication:

Comeau, L. A. and S. E. Campana (submitted May, 2001). Correlations between thyroidal and reproductive endocrine status in wild Atlantic cod. Journal of Fish Biology

Chapter five has been recommended for acceptance (following revision):

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Chapter six has been recommended for acceptance (following revision):

Comeau, L. A., S. E. Campana, and G. A. Chouinard (provisionally accepted, June 2001). Timing of Atlantic cod (*Gadus morhua* L.) seasonal migrations in the southern Gulf of St. Lawrence: interannual variability and proximate control. ICES Journal of Marine Science

CHAPTER ONE

Introduction

VARIOUS PATTERNS OF MIGRATION

Dingle (1995) defined migration as "a specialized behaviour especially evolved for the displacement of an individual in space," and Berthold (1996) suggested that migration patterns change rapidly in response to ecological situations (e.g., global warming) through fast micro evolutionary processes. It is therefore not surprising that migration patterns vary greatly amongst animals, both in terms of distance travelled and periodicity to which they occur. For instance, Chinook salmon *Oncorhynchus tshawytscha* and the European eel *Anguilla anguilla* migrate as far as 4,000 km to spawn, but inevitably do so only once during their life cycle (Sinha and Jones 1975, Allen and Hassler 1986). Conversely, various zooplankton (Papi 1960, Kitting 1985, Carrillo et al. 1989, Balachandran 1991, Dawidowicz and Loose 1992, Miner and Stein 1993, Stewart and Sutherland 1993, Besiktepe and Unsal 2000, Luo et al. 2000) and a number of fish species (De Veen 1978, Frost and McCrone 1979, Willis and Pearcy 1982, Clark and Levy 1988, Clark and Green 1990, Parker 1992, Lough and Potter 1993, Bromley and Kell 1999) carry out vertical migrations over short distances, but repeat them every day.

Seasonal migrations are probably best known in the most conspicuous ungulates such as the Barren Ground caribou *Rangifer tarandus* (McCullough 1985, Bergerud et al. 1990, Heard and Williams 1992), as well as in the avian migrants such as ducks and geese (Orr

1982). In the marine environment, the better-known pelagic species, namely those belonging to the family of Scombridae (e.g., tuna, mackerel, bonito), migrate over hundreds or thousands of kilometres (Castonguay and Gilbert 1995, Belikov et al. 1998, Niiya 1999, Lutcavage et al. 1999), sometimes even across an entire ocean (Mather 1962, Mather et al. 1967). Numerous other animals migrate seasonally over distances that are not so long and impressive by human standards, but are enormous for the means of locomotion of the migrants. These include various insects (Dingle 1972, 1974, Hill and Gatehouse 1993, Tsukada 2000, Zhou et al. 2000), certain marine crustaceans (Herrnkind 1970, Cooper and Uzmann 1971, Haakonsen and Anoruo 1994, Furota et al. 1999), amphibians (Orr 1982, Sinsch 1992, Noli and Grobler 1998, Ponsero and Joly 1998) and small mammals such as bats (Orr 1982, Fenton and Thomas 1985, Rojas et al. 1999), to name but a few.

MECHANISM CONTROLLING MIGRATION

In the interest of conservation, it is becoming increasingly important to have a detailed understanding of the migratory movements of animals. When reflecting on the reasons animals migrate, however, it is useful to distinguish between two levels of causation (Oriens 1962). The adaptive or evolutionary advantages are termed *ultimate* causes, and the cueing parameters are considered *proximate* causes. With respect to the ultimate causes of migration, it is generally agreed that animals migrate between areas where food is abundant and where environmental conditions are favourable for survival and reproduction. The fact that migration is such a common behaviour among avian species indicates that it must be a rather successful strategy (Berthold 1996). For anadromous salmonids, it is believed that the benefits of increased food availability in the oceans outweigh the costs of leaving their freshwater habitat (McLennan 1994). Possible ultimate reasons for the vertical migrations of fish have also been identified. For instance, the diel vertical migration carried out by juvenile cod *Gadus morhua* exposes them to the coolest waters during non-feeding hours, a strategy that likely favours higher growth rates by reducing metabolic costs (Clark and Green 1990, 1991). Similar vertical migrations conducted by zooplankton have been explained as a strategy that minimizes predation risk (Gliwicz 1986, Levy 1990, Ramos and Gonzalez 2000).

Convincing data on proximate causes of migration is available for some terrestrial mammals and avian species. For instance, the ungulates of the Serengeti reportedly migrate in direct response to distant rain (Hairston 1994), which can be seen from afar and which stimulates the growth of savanna vegetation. For temperate bird species, there is mounting laboratory evidence that photoperiod acts as a predominant synchronizer for endogenous circa-annual rhythms linked with migratory restlessness (Berthold 1996). In field investigations, the role of photoperiod has been supported for species such as the spotted flycatcher *Muscicapa striata* and the Bewick's swans *Cygnus bewickii*, which display remarkable year-to-year consistency in the timing of migration (Rees 1989, Kok et al. 1991). Food availability may also be important in some years, since food shortage was shown to increase migratory restlessness in captive spotted flycatchers and garden warblers *Sylvia borin* (Berthold 1996).

Compared to birds, very little is known about proximal causation in the migratory behaviour of fishes. One obvious problem is that there are no established criteria for measuring migratory restlessness in laboratory fishes. Most studies have been aimed at understanding migration-related processes such as smoltification in anadromous salmonids (Baggerman 1957, 1960a, 1960b, 1962, 1963, Saunders and Henderson 1970, Zaugg and Wagner 1973, Wagner 1974, McCleave 1978, Lundqvist 1980, Brauer 1982, Barbour and Garside 1983, Zaugg et al. 1986, Björnsson et al. 1989, Saunders and Harmon 1990, Skilbrei 1991, Duston and Knox 1992, Duncan and Bromage 1998) and metamorphosis in catadromous lampreys (Potter 1970, Manion and Stauffer 1970, Purvis 1980, Cole and Youson 1981, Youson et al. 1993, Fontaine 1994, Cheng and Tzeng 1996). In some studies, certain behaviour patterns, such as changes in salinity preferences (Baggerman 1957, 1960a, 1962, 1963), fluttering or swimming activities (Northcote 1962, Iersel 1970, Woodhead 1970, Olla and Studholme 1978, Castonguay and Cyr 1998), were assumed to be associated with migration onset. However, our knowledge of how well these behaviour patterns reflect migratory restlessness is still highly uncertain.

With respect to field investigations, there are a few quantitative models relating the timing of anadromous and marine pelagic fish migrations to environmental factors, and more specifically to temperature. Using mesoscale time series (7-8 years), Jonsson and Ruud-Hansen (1985) and Bohlin et al. (1993) suggested that the timing of the seaward migration of Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* is largely dependent on water temperature regimes in the weeks preceding the journey. More recently, the seasonal arrival of whale sharks *Rhincodon typus* into coastal waters was correlated with current and water temperature over a six-year period (Wilson et al. 2001). Similarly, landward advections of warm surface waters coincided with the feeding migrations of Atlantic mackerel *Scomber scombrus* to bay areas (Castonguay et al. 1992). There are also indications that the warming of coastal waters may regulate the timing of the shoreward migration of the bluefin tuna *Thynnus thynnus* in the Pacific (Ogawa and Ishida 1989).

ATLANTIC COD SEASONAL MIGRATIONS

Patterns of Cod Migration

Impressive (up to 1,000 km, Woodhead 1975) seasonal migrations are carried out by several marine demersal fish species, including plaice *Pleuronectes platessa* (Gibson 1973), sole *Solea solea* (Wallace 1977, Walker and Emerson 1990, Koutsikopoulos et al. 1995), white hake *Urophycis tenuis* (Kohler 1971), redfish *Sebastes mentella* (Parsons et al. 1978, Morin et al. 1994, Morin et al. 1995), winter flounder *Pseudopleuronectes americanus* flounder (McCracken 1963, Van Guelpen 1979), arrowtooth flounder *Atheresthes stomias* (Rickey 1995), thorny skate *Raja radiata* (Clay 1991), walleye pollock *Theragra chalcogramma* (Radchenko and Sobolevskiy 1993), haddock *Melanogrammus aeglefinus* (McCracken 1963), and Atlantic cod (Huntsman 1932, McKenzie 1934, 1956, Woodhead 1959, Westrheim 1984), among others. Atlantic cod perhaps best exemplifies the migration patterns of demersal fish. Over 50 years of tag and recapture data has shown that cod inhabit deep waters over the continental shelf and slope during winter months; come spring, they migrate to more inshore waters where they reside during the summer. Then, at some point during autumn, they quickly move offshore and ultimately retreat to their overwintering areas over the shelf and slope, thus completing the annual cycle. Such extended and orderly movements are found over the Arcto-Norwegian Shelf (Arnold et al. 1994, Nakken 1994, Godø 1995), the eastern Newfoundland Shelf (Templeman 1979, Lear and Green 1984), the eastern Scotian Shelf (McKenzie 1956), and in the Gulf of St. Lawrence and Cabot Strait region (Jean 1964, Moguedet, 1994). While the exact migration routes are still unclear, the inshore-offshore migration patterns of cod in Canadian waters are illustrated in Figure 1.1.

Mechanism Controlling Cod Migration

Very little is understood about the reasons why cod migrate seasonally. With respect to the ultimate causes, it seems that the spring migration provides cod with good feeding opportunities in shallow inshore areas (Ponomarenko 1996, Lambert and Dutil 1997a, Schwalme and Chouinard 1999, O'Driscoll et al. 2000). In autumn, cod may return offshore to avoid what will ultimately be near freezing waters in their summer habitat. While cod can produce plasma antifreeze glycopeptides (Hew et al. 1981, Fletcher et al. 1987), adult cod do not have the capacity to survive water temperatures below about -1.2°C (Kao and Fletcher 1988).

To insure that the evolutionary benefits of migration outweigh the risks, a safe and reliable control mechanism must be in place so that migratory movements are initiated at

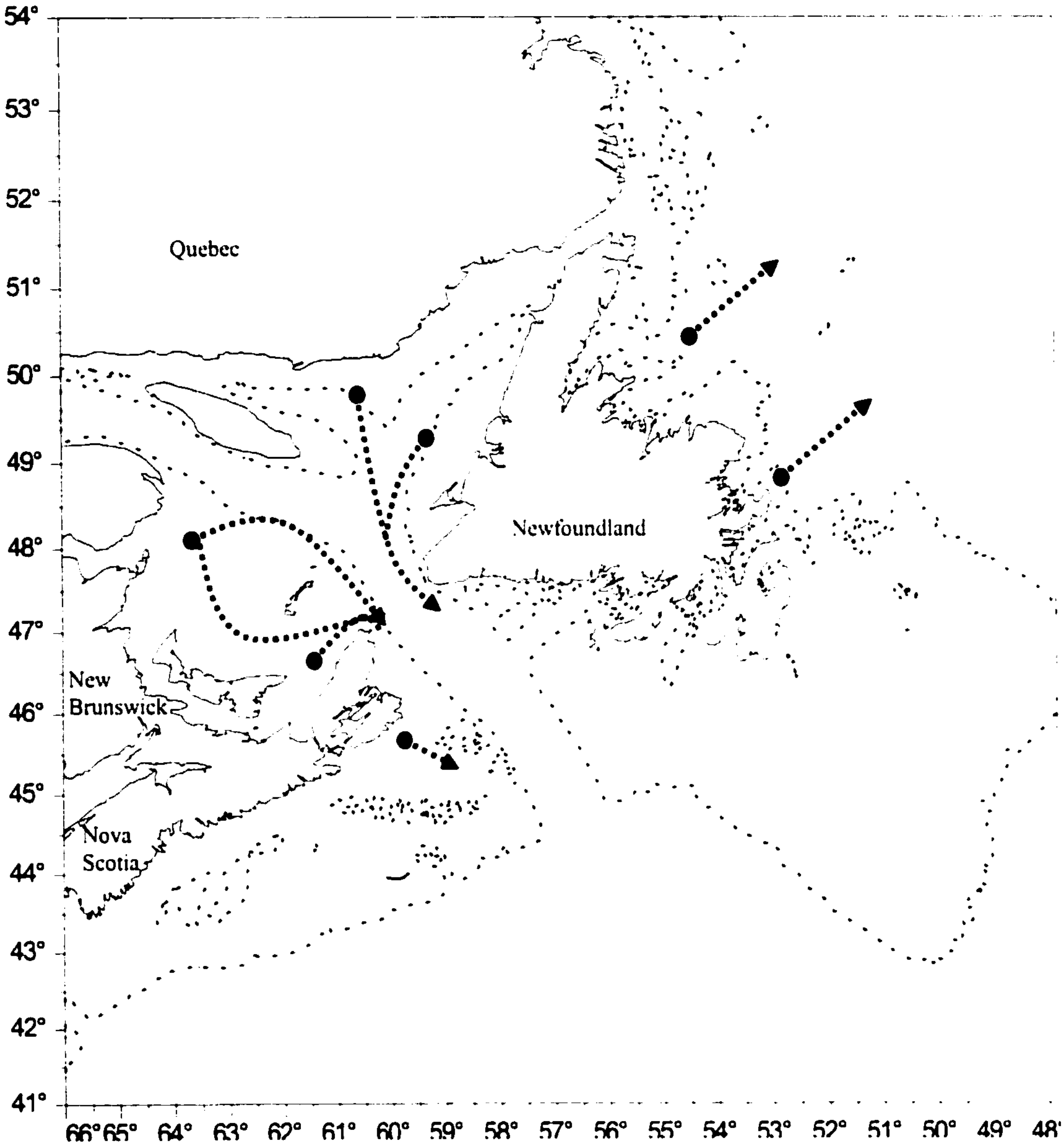

Figure 1.1. Seasonal migration patterns of cod between inshore (summer, ●) and offshore (winter, ▶) areas in Canadian waters (exact routes are not known). Data adapted from McKenzie (1956), Jean (1964), Templeman (1979), Lear and Green (1984), Scott (1988), Moguedet (1992, 1993, 1994), Rose (1993), and Campana et al. (1999). Dotted lines show 200 m depth contour.

the appropriate times within the environmental cycles. Since the cod migrations are synchronized with the seasons, it is tempting to postulate that the cueing mechanism relies upon a specific environmental factor(s). For instance, it has been suggested that the offshore (from continental shelf to slope) migration of demersal species is cued to the cooling of the shelf environment in autumn (Lear and Green 1984, Pinhorn 1984, Moguedet 1994, Shimada and Kimura 1994, Koutsikopoulos et al. 1995). However, a statistical link between the timing of this migration and the cooling of the shelf environment is yet to be reported. As well, the literature makes no apparent mention of the possibility that the offshore migration may be keyed to rising water temperatures—such as those resulting from the annual overturn of the water column. In the Gulf of St. Lawrence, the mixing of warm surface waters with cold bottom waters in autumn causes the temperature in the lower half of the water column to increase from 2°C to about 5°C (where the bottom depth ranges from 35 to 75 m) (Petrie 1990, Swain and Kramer 1995).

If photoperiod is a leading cue to the seasonal migrations undertaken by cod, one would expect that the timing of the events would be constant from year to year. Woodhead and Woodhead (1965) noted that the autumn migration of Barents Sea cod roughly coincided with the autumnal equinox, but regrettably their conclusion was quantitatively weak because only one year of data was available. More recently, Sinclair and Currie (1994) inferred the timing of the autumn and spring migrations of southern Gulf of St. Lawrence cod by investigating port landing statistics across a seven-year series. They suggested that the timing of the autumn migration is rather constant, but that the timing of the spring migration is quite variable. However, the use of port landing statistics remains problematic because the numbers do not account for temporal variation in fishing effort. Investigating the catch per tow figures may more accurately assess the timing of the migrations.

Other plausible but yet untested environmental factors that may cue cod migrations include dissolved oxygen concentrations (D'Amours 1993) and food supply (Templeman 1965). It is well documented that a thermocline at temperate latitudes keeps the water column vertically stratified throughout the summer months and in doing so restricts mixing between the surface and bottom water layers. It is therefore reasonable to believe, without more evidence than reasoning, that limiting oxygen concentrations in near-bottom waters could eventually (i.e., in the autumn) result from the effect of poor oxygen replenishment rates throughout the summer months. With respect to food supply, there is mounting evidence that the spring migration provides individuals with good feeding opportunities in shallow inshore areas (Ponomarenko 1996, Lambert and Dutil 1997a, Schwalme and Chouinard 1999, O'Driscoll et al. 2000). While food may admittedly represent the ultimate reason for migrating shoreward, recent work tends to suggest that the actual migrations can operate without a food trigger. For example, echosounding data revealed that shore-bound eastern Newfoundland cod can migrate with no chief prey (capelin *Mallotus villosus*) in visual sight (Rose 1993). In fact, when encountering capelin, cod may momentarily "veer off-course" in pursuit of it. An alternate hypothesis is that an annual cycle in appetite (see Fletcher 1984, Wootton 1990) regulates migrations to and from feeding grounds.

The environmental cue necessary for cod migration, as yet to be identified, should also bring about a physiological motivation to migrate. According to Woodhead (1959, 1975), the thyroidal system plays a key role in the regulation of cod migration. Woodhead conceptualized that thyroid hormones, which are known to enhance swimming capabilities (Woodhead 1970, Castonguay and Cyr 1998), motivate cod to turn and swim against the prevailing current and towards winter grounds; in keeping with this model, a lowering of thyroid hormone concentrations in spring would allow cod to be transported passively with the current to their summer feeding grounds. A lingering difficulty with this model is that thyroid hormones themselves were never directly measured and correlated with the exact timing of migrations. Woodhead's interpretations were based upon thyroid histological measurements, which are now considered unreliable for detecting short-term changes in the circulating levels of thyroid hormones (Eales and Browns 1993, Cyr and Eales 1996). In addition, there has been growing evidence in recent years that thyroid cycling is functionally linked to the control of gonadal development in salmonid and freshwater species (Cyr and Eales 1996). A similar finding for cod would weaken the premise that the thyroid is primarily concerned with the regulation of migratory behaviour.

Because gonadal steroids are also known to enhance swimming activity (Hoar et al. 1952, 1955, Baggerman 1963, 1966), some investigators have suggested, either implicitly or explicitly, that specific levels of gonadal hormones are required to initiate (spawning) migrations (Scott 1990, Andersen et al. 1991, D'Amours and Castonguay 1992, Wroblewski et al. 1995). Examinations of gonadal tissues in cod suggest that secretory activity does not begin until about two months after the spawning migration (Woodhead and Woodhead 1965), although no attempt has yet been made to correlate the actual levels of gonadal hormones with onset of migratory behaviour. Another argument against the involvement of gonadal hormones was made following the observation that juveniles also migrate seasonally (Woodhead 1975). However, the absence of gonadal stimulation in migrating juveniles may no longer be a valid argument, as more recent hydroacoustic work suggests that juveniles may simply join and follow adults on their migratory journey (Rose 1993).

GENERAL OBJECTIVE AND OUTLINE OF THESIS

The objective of this thesis is to identify the endocrinological and environmental variables cueing the seasonal migrations of southern Gulf of St. Lawrence cod. As a first step in this process, I will provide a description of the annual thyroid hormone cycle in southern Gulf cod, and attempt to identify the factors controlling this cycle (Chapter 2). I will then determine whether changes in the levels of thyroid (and gonadal) hormones are synchronized with the onset of seasonal migrations (Chapter 3). Other roles of thyroid cycles will also be examined, such as their possible involvement in gonadal development (Chapter 4). Base on the idea of tracking thyroid-manipulated cod in their natural environment, I will attempt to create sustained experimental hyper- or hypothyroidism in wild cod (Chapter 5). With respect to the possible influence of environmental factors, migration timing will be compared with changes in water temperature and dissolved oxygen, and also with food intake, energy storage, and prey abundance on summer grounds (Chapter 6). Lastly, I will review my findings and propose a plausible mechanism for the control of cod migrations (Chapter 7).

CHAPTER TWO

Seasonal changes of thyroid hormones in field-collected Atlantic cod (*Gadus morhua* L.) in relation to condition indices, water temperature, and photoperiod

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ABSTRACT

Serum T_4 and T_3 in wild Atlantic cod *Gadus morhua* ranged from 1 to 12 ng ml^{-1} and from 2 to 27 ng ml^{-1} respectively over a 3-year period. In general, the concentrations increased from summer (T_3) or early autumn (T_4) to maxima in mid-winter and declined abruptly during spring. The $T_4 : T_3$ monthly means were lowest in summer and highest in winter. The seasonal patterns of thyroid hormones were weakly correlated with changes in water temperature. However, both T_4 and T_3 co-varied simultaneously with photoperiod. In addition, T_3 was correlated with the hepatosomatic index and condition factor during summer and autumn. It is suggested that the seasonal changes in the release of T_4 from the thyroid were photoperiod-driven, and that the course of T_3 was regulated by the metabolic state of the fish during the somatic growth period.

INTRODUCTION

Although the teleostean thyroid has been researched quite extensively over the past decades, current understanding of its various functions is based almost entirely on freshwater and diadromous species. In these taxa, thyroid hormones promote growth (Donaldson et al. 1979, Higgs et al. 1982) as in the case of higher vertebrates (Gorbman et al. 1983), induce smolting (Boeuf 1993), and stimulate early gonadal development (Cyr and Eales 1996). They may also trigger and sustain upriver and seaward migrations (Godin et al. 1974, Grau et al. 1981, Castonguay et al. 1990, Høgåsen and Prunet 1997).

In marine teleosts, thyroid hormones accelerate larval development (Brown and Kim 1995, Tanaka et al. 1995) and initiate metamorphosis in pleuronectiformes (Inui and Miwa 1985, Inui et al. 1994). However, apart from their role in early ontogeny, little is known about the function of these hormones in marine species, and more precisely about their cyclical patterns in adult marine fishes. The few sporadic reports lead to the conclusion that the seasonal changes in thyroid secretions differ markedly from one species to another. For instance, the concentrations of plasma L-thyroxine (T_4) increased to a single peak during spring in the winter flounder *Pseudopleuronectes americanus* (Eales and Fletcher 1982), whereas the plaice *Pleuronectes platessa* presented a bimodal series with T_4 peaks occurring in both summer and winter (Osborn and Simpson 1978). In contrast, the Atlantic cod *Gadus morhua* showed no obvious T_4 cycle in the laboratory (Cyr et al. 1998), although its thyroid follicular cell height clearly cycled in the wild (Woodhead 1959).

The reasons behind the contradictory accounts of thyroidal cycling are unknown. It could be argued that thyroidal changes are species or population dependent, being sensitive to patterns of food intake and diet composition (Mackenzie et al. 1998), to physical attributes of the habitat (Leatherland 1982, Leloup and De Luze 1985), and to the timing of reproductive events (Cyr and Eales 1996). On the other hand, the thyroidal discrepancies may simply reflect the different study conditions (laboratory *v.* field) or the different capture techniques in field investigations (SCUBA *v.* fishing gear). Some of the divergence between the reports might also reflect the use of different criteria (histological index *v.* blood hormones) for the assessment of thyroidal status (Eales and Brown 1993).

In an attempt to help clarify and understand better thyroid cycling in marine temperate marine fishes, thyroid hormones were studied in field-collected Atlantic cod. The seasonal changes of circulating levels of T_4 and its extrathyroidally-produced derivative 3,5,3'-triiodo-L-thyronine (T_3) were documented over a 3-year period. An attempt is made to identify the environmental and physiological variable(s) that might regulate thyroidal changes, including food intake (stomach fullness), condition, water temperature, and photoperiod. Thyroidal patterns are compared with previous work on cod and other marine teleosts and the functional significance of the thyroid cycles in cod is discussed.

MATERIALS AND METHODS

Sampling area

Atlantic cod were sampled from the southern Gulf of St. Lawrence population on a quasi-monthly schedule between July 1995 and June 1998. The exact sampling area varied according to the seasonal migrations of the cod (Figure 2.1). During the summer – autumn period, samples were collected in traditional fishing areas inside the southern Gulf of St. Lawrence. During the winter and spring, samples were collected in the Cabot Strait area, where southern Gulf of St Lawrence cod overwinter (Paloheimo and Kohler 1968, Campana et al. 1999). Most samples were collected as part of scientific surveys or sentinel fishery expeditions. Mobile gear vessels were employed to catch cod, except off the western coast of Cape Breton Island where baited-longlines were used occasionally. A total of 108 sites were visited during 43 trips. The time interval between individual trips ranged from 1 to 2 weeks during the summer and autumn and from 1 to 2 months during the winter and spring.

Samples attributes and environmental data

The number of fish collected each month ranged from nine to 177 and both sexes were generally well represented (Table 2.1). Except for the June 1998 sample, the average fork length of cod varied from *c*. 49 to 55 cm. Following the collection of blood (see protocol below), the cod were kept on ice and generally dissected within 48 h; otherwise, they were stored frozen at -20°C and dissected after having been partially thawed. The dissections were carried out to determine sex and to measure recent food

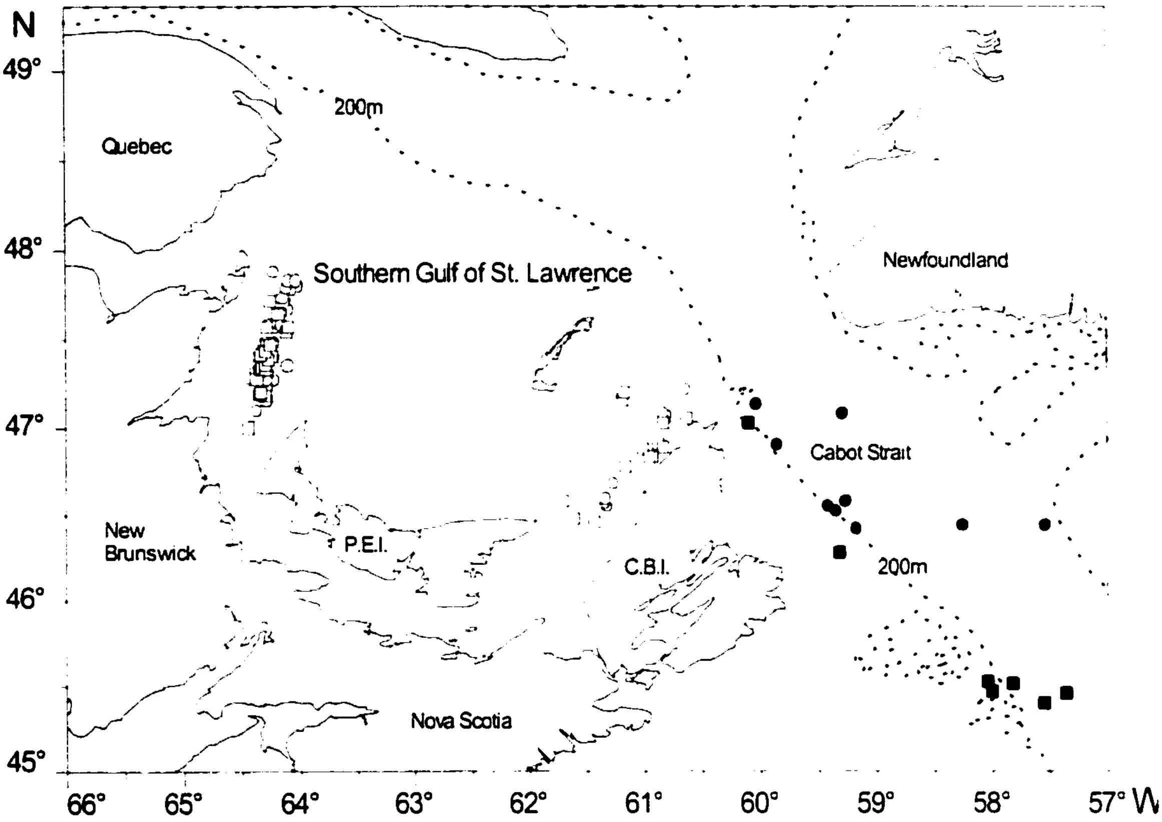

Figure 2.1. Map of the southern Gulf of St. Lawrence area showing capture locations of cod samples during the 1995 – 1998 study period. Samples were collected in winter (●), spring (■), summer (○), autumn (□). The dash lines show the 200 m depth contour. C.B.I., Cape Breton Island; P.E.I., Prince Edward Island.

Table 2.1. Sampling dates, average fork lengths and number of fish included in the assessment of thyroid hormones.

		Length cm		**Number of Fish**		
Year	Month	Mean	(SE)	Male	Female	Total
1995	Jul	48.9	(1.4)	11	7	18
	Aug	55.4	(0.6)	6	11	17
	Sep	52.2	(0.7)	14	18	32
1996	Jan	51.0	(0.8)	23	19	42
	Feb					
	Mar	54.8	(2.3)	3	6	9
	Apr					
	May	55.0	(1.7)	12	9	21
	Jun	53.3	(1.0)	26	37	63
	Jul	51.8	(1.0)	27	33	60
	Aug	54.7	(0.8)	43	43	86
	Sep	51.3	(0.6)	36	41	77
	Oct	54.2	(0.8)	27	43	70
	Nov	49.1	(0.7)	23	39	62
	Dec					
1997	Jan	52.2	(1.2)	22	17	39
	Feb					
	Mar	52.0	(1.1)	18	31	49
	Apr					
	May	49.5	(1.5)	15	15	30
	Jun	53.4	(0.7)	48	49	97
	Jul	54.3	(0.6)	33	64	97
	Aug	55.0	(0.5)	54	63	117
	Sep	54.1	(0.8)	20	17	37
	Oct	53.1	(0.5)	34	30	64
	Nov	50.4	(0.8)	8	19	27
1998	Feb	na	na	na	na	9
	Jun	68.6	(2.9)	8	12	20

intake, hepatosomatic index (I_H), and condition factor. Food intake was reported as the stomach fullness index, representing the weight of the stomach contents as a percentage of the carcass weight (Schwalme and Chouinard 1999). The I_H and condition factor were calculated using somatic weight as in Lambert and Dutil (1997a).

The bottom water temperatures at the time of capture were recorded by securing data loggers (Minilog, Vemco Ltd, Canada) to the fishing gear or by performing CTD casts. The Minilogs had been calibrated at the factory with an estimated accuracy of 0.2°C. In January 1996, bottom temperatures were not recorded for stations where blood was collected. Therefore, the temperatures measured at neighbouring stations (< 10 km) were used, having similar depths and visited during the same cruise. Photoperiod data for the 15th of each month were obtained from the U.S. Naval Observatory (Astronomical Applications Department).

Thyroid hormones

Blood samples were retrieved within *c.* 20 minutes after the fish were brought on board. To reduce sampling time, the aorta was cut rapidly and blood was allowed to flow directly into 7 ml Vacutainer vials. The vials were capped and refrigerated for 12-24 h. Then the serum fraction was then transferred into 2.5 ml cryovial tubes and stored at -72°C until the thyroid hormones were measured by radioimmunoassay (RIA).

Total (protein bound and protein unbound) T_4 and total T_3 were measured using a combined T_4-T_3 RIA (Omeljianuk et al. 1984) and the reagents employed by Cyr et al.

(1998). Barbital buffer (50 mM sodium barbital, 10 mM barbital, pH 8.6) was used for the T_4 segment of the RIA and phosphate buffer (100 mM Na_2 HPO_4, 3mM Na_2EDTA, pH 7.4) for the T_3 segment. T_4 and T_3 (Sigma Chemicals, St. Louis, MO) were diluted serially into 0.1N NaOH to obtain working standards. ^{125}I-T_4 and ^{125}I-T_3 tracers (New England Nuclear, Boston, MA, specific activity 44-46 MBq ug^{-1}) were diluted in 0.1N NaOH until activity was lowered to about 10 000 cpm per 50 µl of solution. T_4 and T_3 antisera (Sigma Chemicals, St. Louis, MO) were diluted in the fitting buffers until binding with the null standard was at 50 – 60%.

A total of 1,143 samples was analysed in duplicate with RIA. The first step in the procedure consisted of introducing 50 µl of each tracer onto G-25 Quik-Sep Sephadex columns (Isolab Inc., Akron, OH) previously equilibrated in 0.1 NaOH. This was followed by the introduction of standards (50 µl of T_4 and 50 µl of T_3) or serum samples (100 µl). Under alkali conditions, thyroid hormones are separated from interfering serum proteins by adsorption onto the Sephadex columns (Alexander and Jennings 1974a, b). Then columns were swirled and the bottom caps removed. After the tracers and standards (or samples) had drained completely into the Sephadex columns, 2 ml of barbital buffer was added to each column and the eluates containing free iodide (^{125}I contamination) and serum proteins were allowed to run to waste. [^{125}I contamination was quantified and corrected for by collecting the eluates from the non-specific binding (NSB) columns.] Then T_4 antiserum (1.0 ml) was pipetted into the columns and the bottom and top caps were replaced to prevent evaporation. After a 4-h incubation at room temperature, 2 ml of barbital buffer was added and the eluates were collected into glass test tubes (12 × 75 mm) for the measurement of the $^{125}I\text{-}T_4$ fractions. Columns were moved over new glass tubes for the second phase of the procedure, where T_3 antiserum (0.5 ml) was added to each column and the caps were replaced. At the end of an overnight incubation, 2 ml of phosphate buffer was pipetted into the columns and the eluates were collected for the determination of the $^{125}I\text{-}T_3$ fractions. Non-specific binding was determined on separate columns that received $^{125}I\text{-}T_4$ and T_3 antiserum only, or $^{125}I\text{-}T_3$ and T_4 antiserum only. Radioactivity was measured using a Beckman Gamma 5500 Counting System. The dilution of pooled serum samples yielded T_4 and T_3 levels close to the theoretical values, and the dilution curves of samples were parallel to the dilution curves of standards. Intra- and interassay coefficients of variation were <10%.

Statistics

Monthly means of thyroid hormones varied in parallel for both genders, with the male-female coefficients of determination being 0.71 for T_4 and 0.90 for T_3. Therefore the average of the male and female monthly means were plotted against time and, in some figures, trend lines were fitted with three-point centred moving averages. However, before running the three-point average function, missing months (eight out of a 30-month series) were replaced with computed values (linear interpolation of two neighbouring values). The trend lines that were generated with this procedure revealed strong cyclic changes over the study period, and autocorrelation function analysis confirmed that series were highly autocorrelated and thus unsuitable for regression analysis. Therefore, correlation analysis was used to quantify the degree to which the variables varied together. No interpolated values were included in the correlation analyses; the analyses were performed on measured data (monthly means) only. A correlation was considered significant when the correlation coefficient (r) was greater than twice its standard error.

In addition, cross-correlation function (CCF) analysis was used to examine whether thyroid series lagged behind environmental or physiological changes, a situation that might be expected if changes in the concentration levels of circulating hormones were induced by the regulatory variable. Because CCF analysis is appropriate only for observations at equally spaced time intervals, missing monthly means were replaced with linear interpolated values. CCF analyses were performed on continuous time series covering the period July 1995 to November 1997, with the exception of those involving condition indices, which were limited to the period June 1996 to November 1997 because it was judged that too many data points were missing during the winter and spring of 1996. The results of each CCF analysis were inspected to determine the lag (0-6 months) at which the maximum correlation coefficient occurred between the two variables. Then the previously interpolated values were removed and the two variables correlated at the fitting lag. Therefore, although the identification of the lag time was based on both measured and interpolated values, the correlation coefficients themselves were computed using measured data only.

RESULTS

Thyroid hormones were examined in relation to the size of fish sampled. For each sampling cruise, the concentrations of thyroid hormones in individuals were regressed against their fork length. Significant, although generally weak, relationships were found in only 7% of cruises for T_4 and 16% for T_3. Moreover, the relationships did not appear at a particular season during the year and the slopes were manifested in both directions, indicating that hormone levels were not influenced by size of fish.

Serum T_4 levels ranged from *c.* 1 to 12 ng ml^{-1} (monthly means), whereas serum T_3 varied from *c.* 2 to 27 ng ml^{-1}. Both hormones varied similarly across seasons, with a single peak occurring annually (Figure 2.2). In general, the hormones increased from summer (T_3) or early autumn (T_4) to maxima in mid-winter and declined abruptly during spring. The complete T_4 and T_3 series were introduced into a CCF analysis to determine the extent to which the two hormones co-varied. The peak correlation coefficient appeared when the T_3 cycle was set ahead of the T_4 cycle by 1 month. However, after removing the July – September period from the series, the two hormones co-varied simultaneously, indicating that the T_3 lead over T_4 was limited to the summer period. The $T_4 : T_3$ monthly means were lowest in summer (Figure 2.3).

The above seasonal changes are strikingly similar to the seasonal changes in the thyroid cell height previously reported for the Barents Sea cod (Woodhead 1959) (Figure 2.4). A CCF analysis indicated that the T_4 and histological index co-vary simultaneously. Moreover, a correlation analysis showed that the coefficient of determination between T_4 and the histological index was high ($r^2 = 0.84$). However, the extrathyroidally produced T_3 does not co-vary as well with the histological index, the coefficient of determination being 0.58.

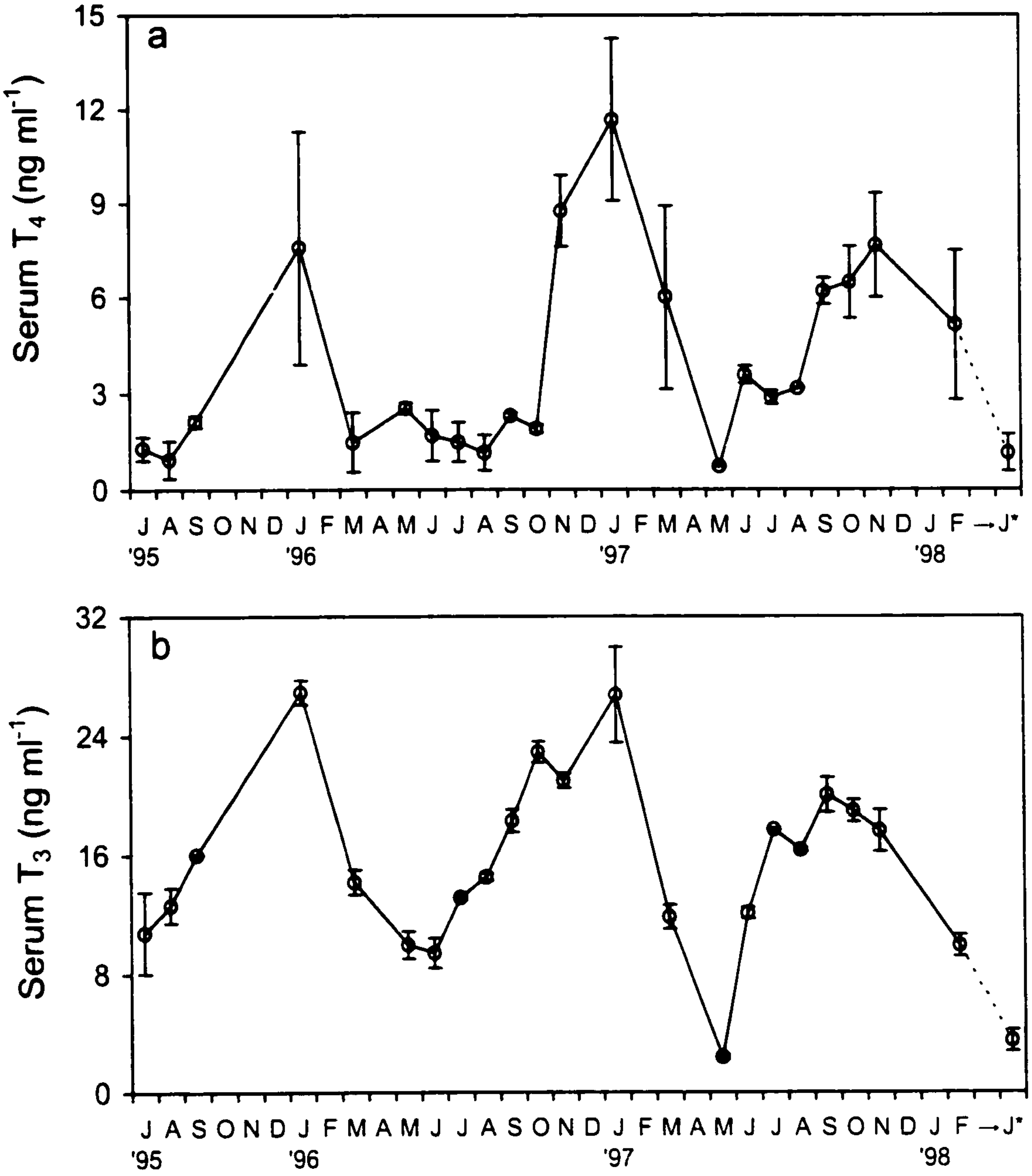

Figure 2.2. Seasonal changes in serum L-thyroxine (T_4) (a) and triiodo-L-thyronine (T_3) (b) levels in southern Gulf of St. Lawrence cod. Data points represent averages of male and female monthly means. Error bars show SE. J* is June 1998.

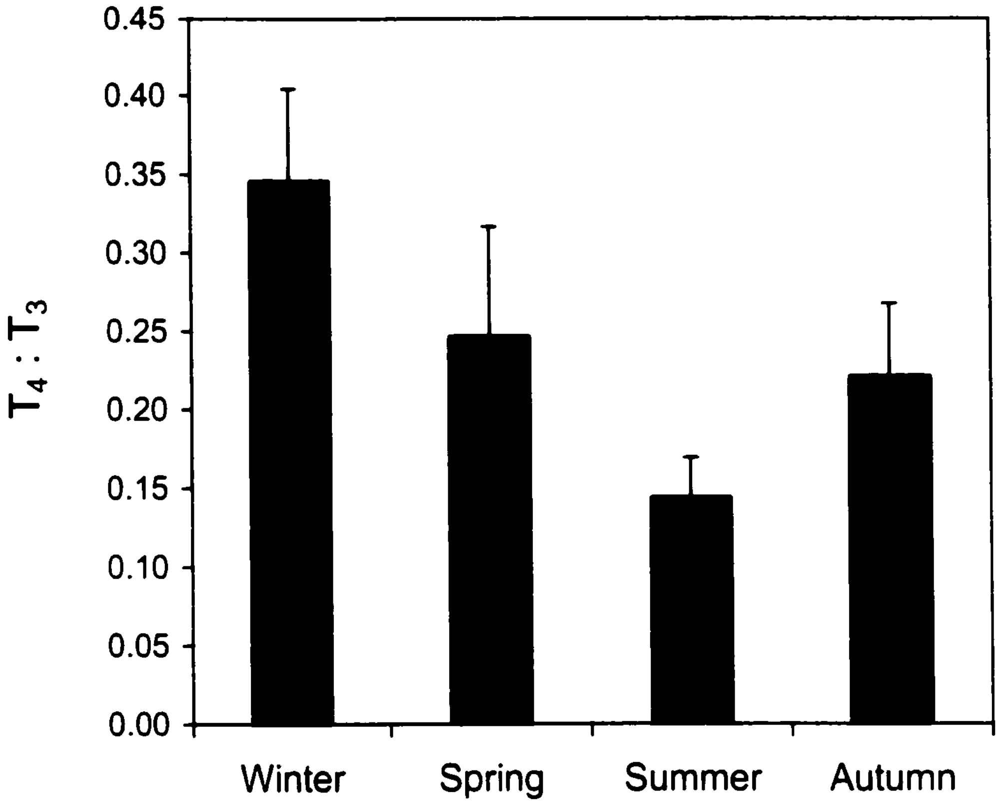

Figure 2.3. Seasonal averages of T_4 : T_3 molar ratios for southern Gulf of St. Lawrence cod. The averages are based on monthly means. Error bars show SE.

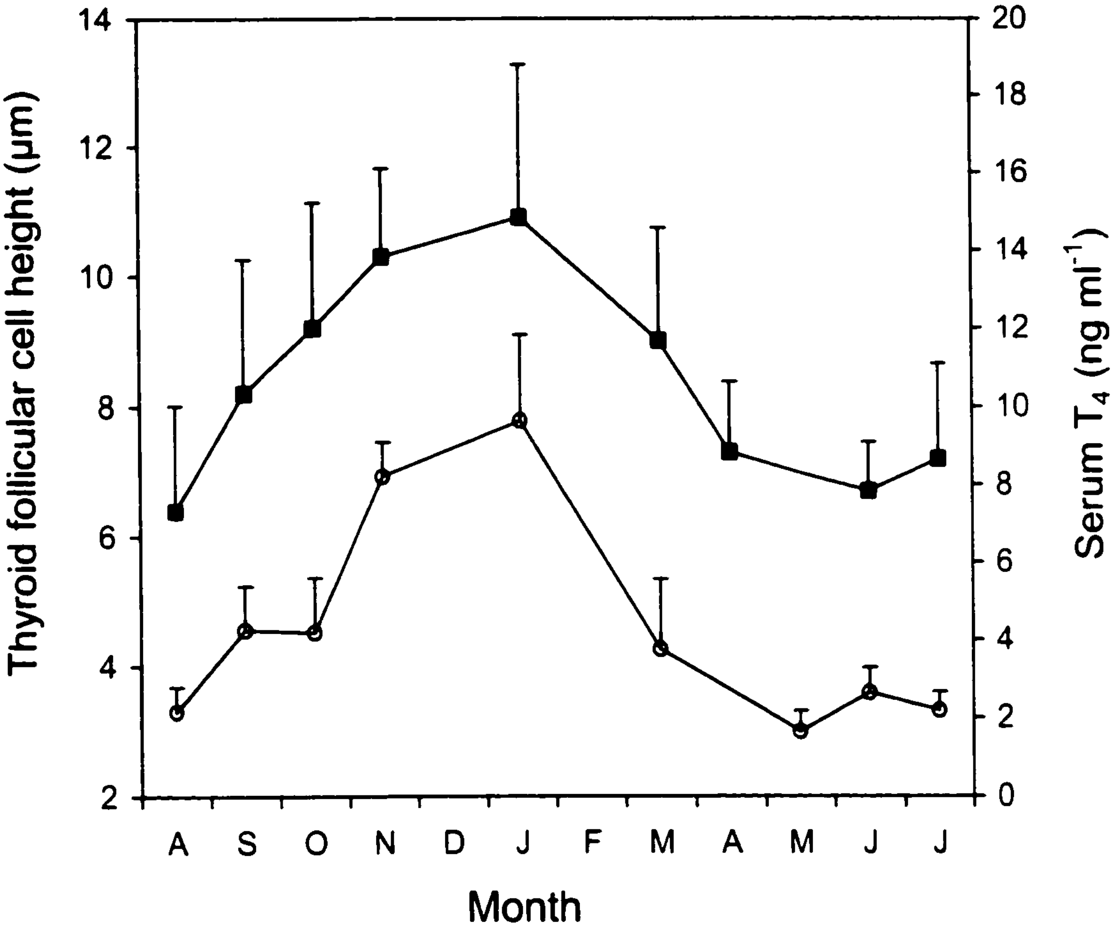

Figure 2.4. Seasonal changes in thyroid follicular cell height in Barents Sea cod (August 1956 to July 1957—data from Woodhead, 1959) (■) and serum T_4 in the southern Gulf of St. Lawrence cod (○). T_4 represents average of 1996 and 1997 series. Error bars represent SE.

Thyroidal cycles, in relation to bottom water temperature, photoperiod, and condition, were considered next. In simple correlation analyses, T_4 was weakly correlated (r^2 = 0.28) with temperature whereas T_3 was not correlated with temperature (Table 2.2). Moreover, a visual examination of the time series suggested that T_4, and particularly T_3, preceded the temperature cycle [Figure 2.5(a, b)]. Such a lead by thyroid hormones is counter-intuitive to a temperature control mechanism. So CCF analyses were used to examine the possibility that the thyroid hormones lagged behind temperature. The hormones were strongly correlated with temperature only when a four to five month lag was applied (Table 2.2). Also, the correlations at these lags were negative. In contrast, thyroid hormones and the thyroid histological index (data taken from Woodhead 1959) were correlated simultaneously and positively with night-length (r^2 = 0.58 – 0.92) [Figure 2.5 (c, d)]. CCF analyses failed to improve the correlations with night-length. As regard condition indices, T_4 and T_3 were correlated negatively with the stomach fullness index [Figure 2.6(a,b)]. A positive and strong correlation was found when T_4 lagged behind the stomach fullness index by five months [Figure 2.6(a)], or the condition factor by one month [Figure 2.6(e)]. T_3 in turn co-varied simultaneously and positively with the I_H [r^2 = 0.43, Figure 2.6(d)] and condition factor [r^2 = 0.52, Figure 2.6(f)], although only during the June to October period.

Table 2.2. Correlation and cross-correlation function (CCF) analyses between environmental and thyroid data presented in Figure 1.5, and between condition and thyroid data presented in Figure 1.6. r^2 = coefficient of determination. The thyroid follicular cell height data were taken from Woodhead (1959).

		Correlation		CCF		
Variable 1	Variable 2	r^2	r^a	Lag^b	r^2	r^a
Environment						
Temperature	T_4	0.28*	+	5	0.85*	–
	T_3	0.13		4	0.59*	–
Night-length	T_4	0.59*	+			
	T_3	0.58*	+			
	Follicular cell height	0.92*	+			
Condition						
Stomach fullness	T_4	0.44*	–	5	0.70*	+
	T_3	0.31*	–			
I_H	T_4	0.03				
	T_3	0.43$^{*\ JO}$	+			
Condition factor	T_4	0.30$^{*\ JO}$	+	1	0.47*	+
	T_3	0.52$^{*\ JO}$	+			

[a] Sign associated with correlation coefficient r.

[b] Number of months that thyroid cycles lagged behind environmental or condition cycles.

[*] Significant correlation ($r > 2$ S.E.).

[JO] Analysis including the June to October (1995 – 1997) period only.

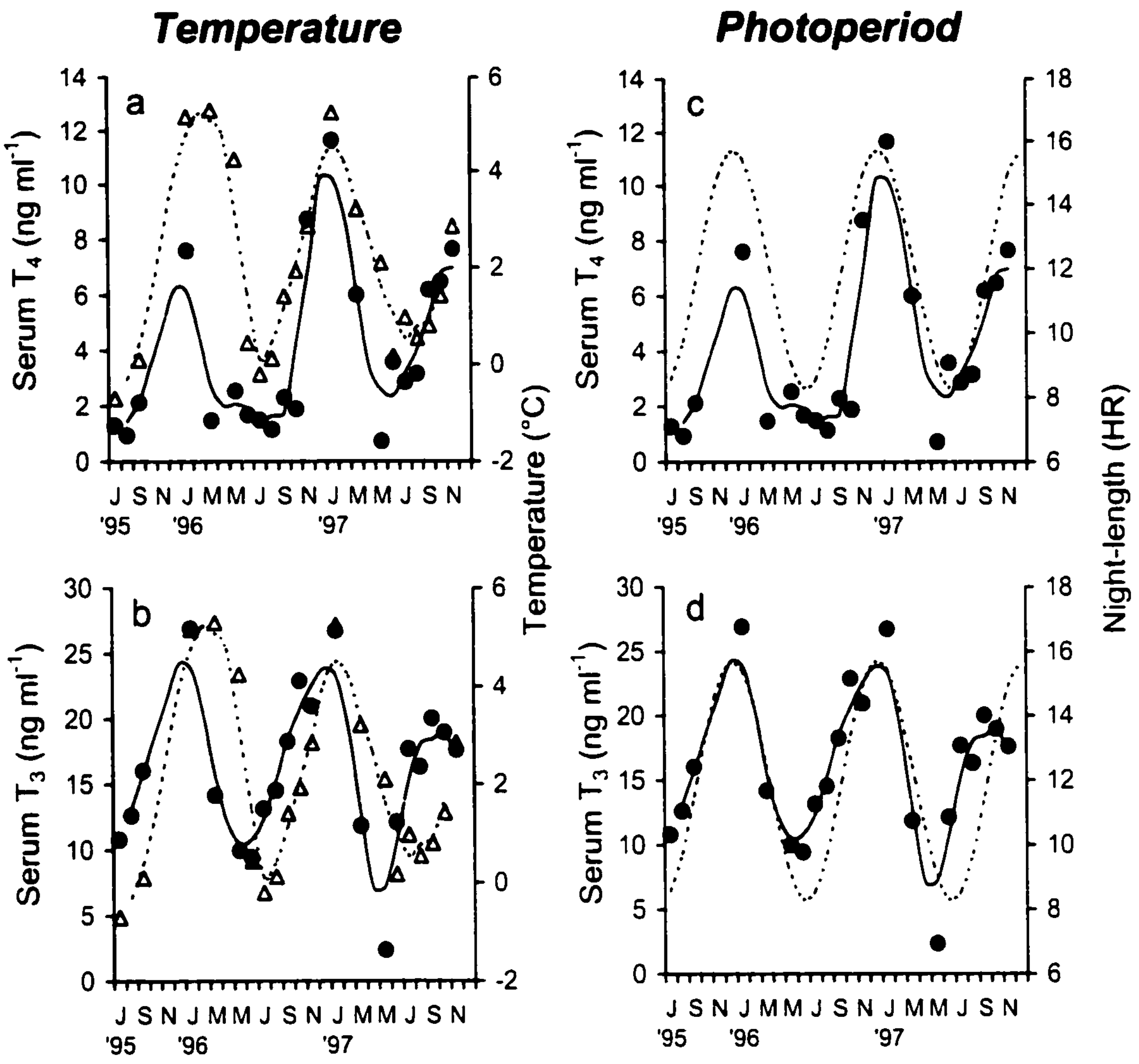

Figure 2.5. Cycling of thyroid hormones in relation to bottom water temperature and night-length. ●, Monthly means of either T_4 [(a), (c)] or T_3 [(b), (d)]; Δ, bottom water temperature. Trend lines show three-point centred moving averages for hormones (—) and temperature (---) series. Night-length dotted lines (under Photoperiod) simply connect data points on 15th of each month.

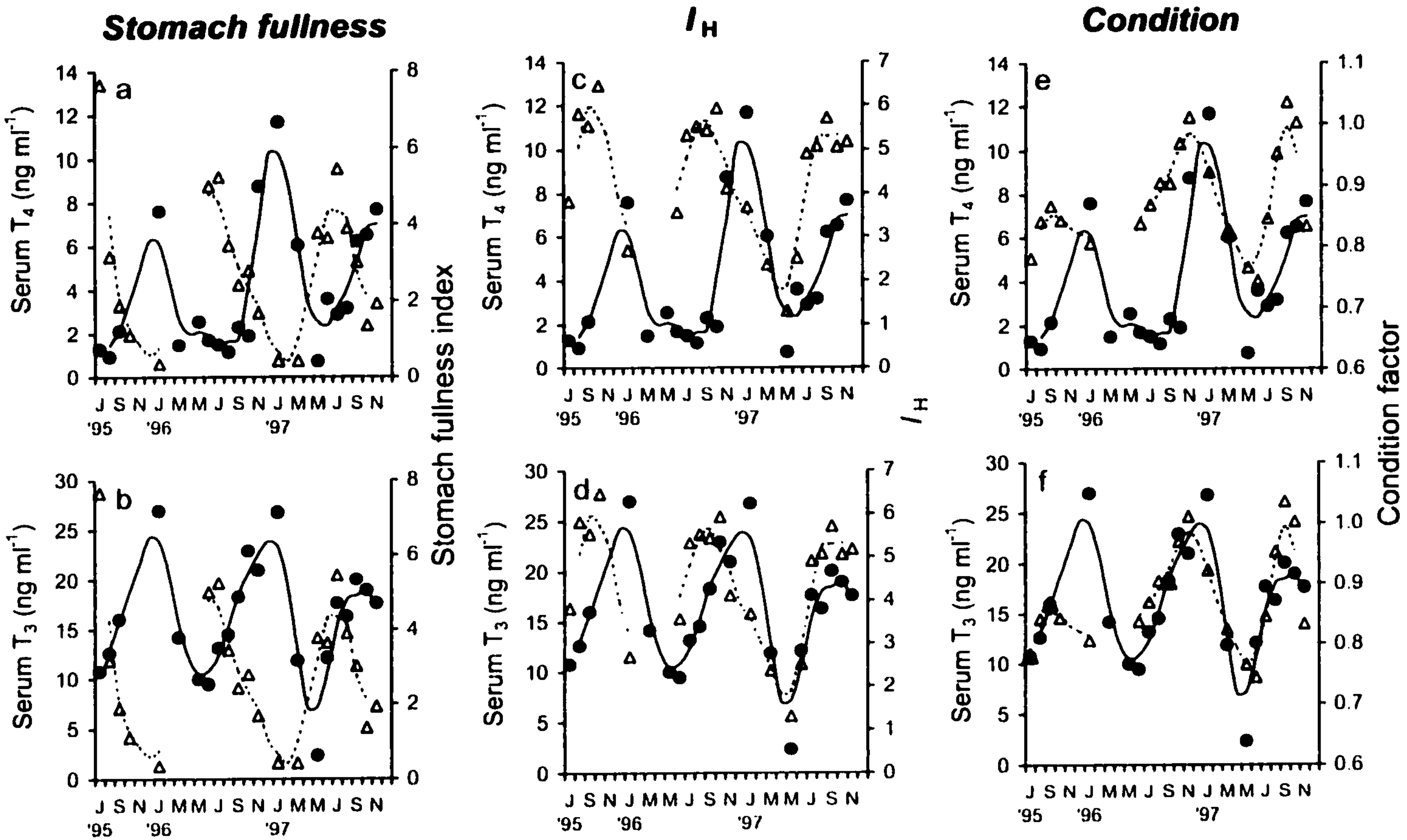

Figure 2.6. Cycling of thyroid hormones in relation to the stomach fullness index, I_H, and condition factor. ●, Monthly means of either T_4 [(a), (c), (e)] or T_3 [(b), (d), (f)]; Δ, stomach fullness index (a), (b), I_H (c), (d), condition factor (e), (f). Trend lines show three-point centred moving averages for hormones (—), stomach fullness index (--- under Stomach fullness), I_H (--- under I_H), and condition factor (--- under Condition). Dotted trend lines were not plotted for the winter of 1996 because too many data points were missing.

DISCUSSION

Description of thyroid cycles

Serum T_4 in wild cod varied seasonally from *c.* 1 to 12 ng ml^{-1} (monthly means), a range which is comparable to those of other demersal species sampled after catch: T_4 varied from *c.* 2 to 16 ng ml^{-1} in the winter flounder (Eales and Fletcher 1982), and from *c.* 3 to 13 ng ml^{-1} in the plaice (Osborn and Simpson 1978). In contrast, levels of the extrathyroidally produced T_3 were more variable and seemingly higher than those reported for flounder and plaice. T_3 ranged from 2 to 27 ng ml^{-1} in cod, compared with *c.* 2 to 19 ng ml^{-1} in flounder (Eales and Fletcher 1982) and 3 to 9 ng ml^{-1} in plaice (Osborn and Simpson 1978). Also, unlike flounder and plaice, cod exhibited T_3 levels that were consistently higher than T_4 levels. Cyr et al. (1998) found similar $T_4 - T_3$ concentration differences for laboratory male cod (females unavailable). Therefore, the study on both sexes reinforces the suggestion that cod have a remarkable capacity for synthesizing T_3.

Serum T_4 and T_3 in field-collected cod followed a consistent annual pattern with a single peak occurring in winter. A short-lasting but recurring divergence in the courses taken by the two hormones was noted: T_3 levels began rising during summer whereas T_4 levels remained low until at least September. Different results pertaining to seasonal changes of thyroid hormones were obtained under laboratory conditions (Cyr et al. 1998), where male cod displayed three annual T_3 peaks (October, February, and May) and no obvious T_4 cycle. The exact reasons for these contrasting results cannot be answered, although satiation feeding (Mackenzie et al. 1998) or stress associated with captivity and stocking density (Laidley and Leatherland 1988) may have distorted the natural course of thyroidal changes in laboratory cod. Similarly, it is recognized that the cod sampled at sea were stressed to some extent by capture and the sudden removal from their deep-water environment. The total time between the capture near sea bottom and the collection of blood samples was <90 minutes for most individuals. In comparison, significant changes in plasma T_4 levels in rainbow trout *Oncorhynchus mykiss* were detected only 8 h after treatment with a handling and asphyxiation stressor (Leatherland 1985) and 2 h following deliberate disturbance (Himick and Eales 1990). In another study on rainbow trout, Brown et al. (1978) found no changes in plasma T_4 30 min following physical injury; a peak in T_4 was recorded only 2 h following injury. Plasma T_3 levels were unaltered by the handling/asphyxiation stressor (Leatherland 1985) and deliberate disturbance (Himick and Eales 1990), and were mostly unresponsive to physical injury (Brown et al. 1978). Eales and Fletcher (1982) came to similar conclusions after examining the flounder's thyroid responsiveness to a physical stressor (individuals kept upside down in a confined area for 1 h). Therefore, the cycles of thyroid hormones in this study probably reflect natural changes given the relatively short period of time associated with the capture event.

The present data were correlated unexpectedly with those of another field investigation, in which the thyroid follicular cell height was monitored as a proxy for thyroid hormone production in Barents Sea cod (Woodhead 1959). While histological indices are considered unreliable for detecting short-term changes in thyroidal status, they have been successful in detecting chronic changes in plasma T_4 in salmonids (Eales and Brown 1993). In the present study, the correlation between the histological index and hormones was particularly strong for T_4 ($r^2 = 0.84$), which is consistent with the view that the thyroid secretes primarily T_4. Therefore, the southern Gulf of St Lawrence and Barents Sea cod probably share a similar annual cycle of thyroid secretions. This result is of particular interest since the two cod populations inhabit very different oceanographic systems, temperate and sub-arctic, on different sides of the Atlantic. The parallel changes in thyroid function for both populations suggest that the thyroid is an important regulator of physiological processes in cod.

However, the seasonal course of thyroidal secretions seems to differ among the few marine fishes investigated to date. T_4 followed different annual patterns in the winter flounder (Eales and Fletcher 1982), plaice (Osborn and Simpson 1978), and cod (this study). Nevertheless, similarities between the marine fishes emerge when thyroidal status is judged by the $T_4 : T_3$ ratio. This ratio is highest during winter (cod, plaice) or spring (flounder), and lowest during summer (cod, plaice, flounder) (Osborn and Simpson 1978, Eales and Fletcher 1982, this study). In other teleosts, the $T_4 : T_3$ (or $T_3 : T_4$) ratio has also been found to be a sensitive index of thyroidal changes that were not necessarily detected by T_4 or T_3 levels alone (Eales and Brown 1993, Björnsson et al. 1998). The ratio is capable of signalling a shift in the equilibrium between T_4 and T_3 serum pools and, conceivably, also between T_4 and T_3 production or degradation rates. At present, the few data available suggest that the summer $T_4 : T_3$ disequilibrium is a common thyroidal feature among temperate marine teleosts. The likely environmental and physiological factors regulating the course of T_4, T_3, and $T_4 : T_3$ ratios are identified below.

Control of thyroid cycles

Swift (1960) suggested that the seasonal changes in thyroidal activity in cold-water teleosts are regulated primarily by water temperature. However, more recent data concerning the influence of temperature on blood circulating levels of thyroid hormones are conflicting. On the one hand, plasma levels of thyroid hormones were sensitive to temperature in starved eels (Leloup et al. 1984, Leloup and De Luze 1985) and also in trout fed specific diets (Leatherland et al. 1977, 1980). On the other hand, levels of thyroid hormones were not altered by temperature in trout fed common diets (Eales et al. 1982, 1986) or cod fed to satiation with capelin *Mallotus villosus* (Cyr et al. 1998). For cod sampled in their natural habitat (this study), T_4 was weakly correlated ($r^2 = 0.28$) with spatio-temporal changes in water temperature. Strong and negative correlations were found when thyroid hormones lagged temperature changes by 4-5 months, suggesting, for instance, that peak winter temperatures resulted in reduced thyroidal status in spring or summer. These findings make it unlikely that thyroid cycles in cod were entrained by temperature.

A temperature effect on thyroidal status might be detected through the $T_4 : T_3$ ratios. Elevated water temperatures in summer were suspected of lowering of this ratio in rainbow trout (Osborn et al. 1978), plaice (Osborn and Simpson 1978), and winter flounder (Eales and Fletcher 1982). Eales and Fletcher (1982) suggested that the lowering of the $T_4 : T_3$ ratio in summer could be attributed in part to elevated temperatures acting upon T_4 degradation rates and T_4 to T_3 conversion rates. This interpretation is consistent with laboratory work showing that temperature tends to increase T_4 degradation rates (Eales et al. 1982), *in vivo* T_4 to T_3 conversion rates (Eales et al. 1982), and *in vitro* hepatic T_4ORD (T_4 outer-ring deiodinase – responsible for T_4 to T_3 conversion) activity (Johnston and Eales 1995) in the rainbow trout. Yet, in the present study, such effects of temperature were not noticeable through the seasonal changes in the $T_4 : T_3$ ratio. Although there was a significant lowering of the ratio during summer, as reported for other temperate species, cod inhabits colder waters in summer than in winter [Swain et al. 1998, see also Figure 2.5(a)]. In keeping with this observation, it seems unlikely that habitat temperature acting on T_4 degradation rates or T_4 to T_3 conversion rates lowered the $T_4 : T_3$ ratio during summer. Instead, cold water in summer might have lowered the $T_4 : T_3$ ratio by reducing T_4 secretion rates and possibly also T_3 clearance and tissue uptake rates. However, there is no support yet for the hypothesis that temperature regulates T_4 release in cod (Cyr et al. 1998, this study), and the data as to whether the effects of temperature on T_3 clearance rates can modify T_3 levels in the blood compartment are conflicting (Leloup et al. 1984, Leloup and De Luze 1985, Eales et al. 1986). In summary, there was no obvious relationship between habitat temperature and thyroid cycling (T_4, T_3, $T_4 : T_3$), and thus it is suggested that the annual thyroid cycles in wild cod are not temperature-driven.

The effects of photoperiod on the thyroidal system of marine teleosts are not documented, but short day-lengths or complete darkness tend to stimulate the thyroid in freshwater and euryhaline species (Eales 1979, Brown 1988). In these taxa at least, short day-lengths may decrease the sensitivity of the hypothalamic-hypophyseal negative feedback mechanism, thereby enabling prolonged thyroidal responses to the thyroid-stimulating hormone (Brown and Stetson 1985, Grau et al. 1985). Strong field correlations in the present study support such a thyroid responsiveness model: the night-length was correlated positively and simultaneously with the thyroid follicular cell height ($r^2 = 0.92$) (data taken from Woodhead 1959) and the major thyroid secretion (T_4) ($r^2 = 0.59$). These correlations suggest that photoperiod is the main entrainer of thyroid secretions in cod. Woodhead and Woodhead (1965) came to a similar conclusion after reviewing their histological data for Barents Sea cod. However, they suggested that the activation of the thyroid is cued to the autumn equinox and not to the rate of change of photoperiod. In agreement with this model, the present T_4 series did not show any increasing trend before September. However, once activated at the autumn equinox it seems that the thyroid in cod would respond to the rate of change of photoperiod, as suggested by present field correlations.

There was a positive correlation between night-length and T_3 ($r^2 = 0.58$), which suggest that photoperiod might have acted also on the peripheral control of thyroidal status. As the majority of T_3 in teleosts is thought to derive from T_4ORD activity in peripheral tissues (Eales and Brown 1993), the field correlation suggests that photoperiod acted specifically on the T_4 deiodinating pathways. Unfortunately, tissue T_4ORD activity was not monitored and so it cannot be verified that T_4ORD varied in concert with photoperiod in cod. However, Cyr et al. (1998) exposed cod to a naturally simulated photoperiod but constant temperature (2-4°C) and feeding regime, and reported that hepatic T_4ORD activity was about three-fold higher in February than in June. While this experiment was not designed specifically to test the effects of photoperiod, its results, together with the present night-length *v.* T_3 field correlation, raise the question as to whether photoperiod entrained an underlying rhythm of T_4ORD activity. Alternatively, it is possible T_4ORD activity was not affected by photoperiod directly, but by T_4 availability. In this case, the diminishing daylength in winter would have increased T_4 levels (see above), which in turn would have enhanced T_4ORD activity and thus T_3 levels in circulation. However, a photoperiod control mechanism of T_3, either direct or indirect (*via* T_4), does not conform to the present summer results. Indeed, it appears that T_3 preceded night-length during summer, particularly in 1997 [Figure 2.5(d)]. The T_3 lead over night-length, although small (about one month), implies that the T_3 producing mechanism was insensitive to photoperiod at this time of year. Also, low T_4 levels during summer are inconsistent with the idea that T_4 increased T_4ORD activity and T_3 levels in circulation. Therefore, the data indicated that photoperiod might have some influence upon T_3 production in cod, except during the summer period where the potential effects of photoperiod seemed overridden by other factors.

The thyroid cycles in cod might have been influenced by marked seasonal changes in feeding intensity. In aquaculturally important species, thyroid hormones have been related positively to food intake, as demonstrated by various experimental designs (food deprivation, refeeding, different rations) (Eales 1988, Mackenzie et al. 1998). For cod, thyroid hormones were negatively correlated with the stomach fullness index, which is remarkably inconsistent with experimental evidence. A positive correlation was found only when the T_4 cycle lagged behind the stomach fullness index by 5 months. Thus there was no evidence that the thyroidal system in cod responded instantaneously to the seasonal changes in feeding intensity. However, it seems that T_3 levels were influenced by changes in condition during summer and autumn (June to October). At this time, T_3 co-varied simultaneously with both the I_H ($r^2 = 0.43$) and the condition factor ($r^2 = 0.52$). The difference between the two condition indices may be related to their capacity in reflecting changes in T_4ORD activity in cod. It is possible that the condition factor, which yielded the highest coefficient of determination, reflected changes in total (all tissues) T_4ORD activity. The I_H in turn may be correlated with hepatic T_4ORD activity mostly. In keeping with these assumptions, one might conclude that the liver alone explained a substantial proportion of the variation in T_3 levels. As T_4ORD activity was not monitored, it cannot be confirmed that the recorded seasonal changes in the condition indices were accompanied by parallel changes in the cod's potential for generating T_3. Nonetheless, it is concluded that the thyroidal trends in wild cod were in broad agreement with the emerging view that the primary control mechanism for teleostean T_3 is peripheral, with the liver playing a central role (Farbridge et al. 1992, Eales and Brown 1993, Morin et al. 1993, Van der Geyten et al. 1998).

Together the above correlations lead to the suggestion that the diverging courses taken by T_4 and T_3 during summer, and hence the lowering of the $T_4 : T_3$ ratios, were related to increased day-length and condition. The overall impression is that long day-lengths reduced T_4 secretion at the same time as the rising metabolic state of cod increased T_3 production. Further, because T_3 is derived from T_4 (Eales and Brown 1993), it is possible that enhanced T_3 production during summer also contributed to the lowering of serum T_4 levels (i.e., on top of the thyroid's low T_4 release). This interpretation differs from the one presented for the trout (Osborn et al. 1978) and winter flounder (Eales and Fletcher 1982), where temperature was identified as one likely cause for the summer disequilibrium between T_4 and T_3. Present findings are more consistent with those of Osborn and Simpson (1978), who suggested that the plaice's thyroid might be more sensitive to changes in day-length than temperature.

Functional role of thyroid cycles

Thyroid hormones are widely recognized as permissive growth inducers in aquaculturally important teleosts (mainly salmonids) (Donaldson et al. 1979, Higgs et al. 1979, 1982). This growth-promoting role has yet to be validated for adult marine fishes displaying marked seasonal changes in feeding intensity and growth patterns. Recent data on the seasonal changes in carcass weights for the southern Gulf of St. Lawrence cod (Schwalme and Chouinard 1999) indicate that maximal growth rates occur between June and October in this population. In comparison, serum T_4 levels in the present study started increasing in autumn only. However, T_3 levels started increasing during summer well in advance of T_4. The closer association between T_3 and maximal growth rates in cod is consistent with evidence that T_3 is more effective than T_4 in promoting fish growth (Higgs et al. 1979). It is equally interesting that serum T_3 levels, in agreement with the hormone's stimulating effect on early ovarian development in trout (Cyr and Eales 1996), started increasing immediately after spawning (early summer, Schwalme and Chouinard 1999). Thus the promotion of early gonadal development is a second plausible reason for the increasing T_3 levels in cod during summer. In contrast, the lowering of T_3 levels in the final stages of gonadal development (spring, Schwalme and Chouinard 1999) is consistent with the view that thyroid hormones are unessential to the latter stages of the reproductive events (Cyr and Eales 1996). Moreover, by curtailing somatic growth, a suppressed thyroidal status in spring may allow the remaining metabolic reserves to be directed towards continued gonadal growth.

While T_3 may be involved in the stimulation of somatic and early gonadal growth, T_4 could function as an endocrinological trigger and metabolic stimulus to the lengthy (*c.* 500 km – Hanson 1996) autumn migration. Until now support for this hypothesis has been based on the cycling of the thyroid follicular cell height in migratory Barents Sea cod (Woodhead 1959, 1975, Woodhead and Woodhead 1965), and also on the observation of increased locomotor activity in T_4-injected cod (Woodhead 1970, Castonguay and Cyr 1998). Present work has provided the first documentation of an autumnal T_4 increase inside a highly migratory cod population, adding support to the view that T_4 might be involved in the physiological mechanism triggering migration.

In conclusion, blood-circulating levels of T_4 in cod were found to be similar to those previously reported for plaice (Osborn and Simpson 1978) and winter flounder (Eales and Fletcher 1982), although T_3 levels were comparatively elevated in cod. Both hormones followed a consistent annual pattern with a single peak occurring in winter. A comparative analysis with previous work suggests that: (1) thyroid cycling varies identically in cod populations and (2) at least one thyroidal feature, the summer disequilibrium between T_4 and T_3 circulating levels, may be common in temperate marine teleosts. Spatio-temporal changes in water temperature provided no satisfactory explanation for thyroidal patterns. However, it seems that the metabolic condition of cod regulated T_3 levels during summer and autumn. These results imply that the seasonal stimulation of somatic and gonadal growth by T_3 is determined ultimately by food resources, and not by variations in water temperature. Photoperiod was identified as the prime candidate for entraining the annual cycle of T_4 release from the thyroid. There are also indications that the thyroid's response to photoperiod might be triggered by the autumn equinox. Such a mechanism would lead to a synchronicity throughout the Northern Hemisphere of physiological and behavioural processes under the influence of T_4, such as perhaps the onset of seasonal migrations.

CHAPTER THREE

Timing of Atlantic cod (*Gadus morhua* L.) seasonal migrations in relation to serum levels of gonadal and thyroidal hormones

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ABSTRACT

Serum testosterone, estradiol-17ß (E_2), L-thyroxine (T_4), 3,5,3'-triiodo-L-thyronine (T_3), and free (protein unbound) T_3 concentrations were examined in relation to the extensive (up to 650 km) seasonal migrations undertaken by southern Gulf of St. Lawrence cod (*Gadus morhua*). Testosterone levels were low as the stock moved out of the Gulf of St. Lawrence in autumn, and increased only during the final stages of the return migration in spring. E_2 in females peaked near the onset of the spring migration, and remained elevated until this migration was completed in early summer. However, I suggest that the changes in E_2 levels are functionally tied with egg development and not with spring migratory behaviour. Free T_3 showed no consistent pattern in relation to seasonal migrations, whereas T_4 and T_3 levels increased at the start of the autumn migration. I conclude that thyroid hormones may facilitate the onset of the autumn migration by enhancing metabolism, sensory physiology and swimming capacity.

INTRODUCTION

Atlantic cod (*Gadus morhua*) populations generally move from coastal to offshore areas in autumn, overwinter in deep slope waters, and return to more inshore areas in the spring. Such orderly and extended (up to 1,000 km, Woodhead 1975) seasonal migrations are now recognized for many of the world's most commercially-important cod populations, such as the Arcto-Norwegian and Barents Sea cod (Harden Jones 1968, Woodhead 1975), West Greenland cod (Harden Jones 1968, Hovgård and Christensen 1990), Newfoundland and Labrador cod (Templeman 1979, Lear and Green 1984), eastern Scotian Shelf cod (McKenzie 1956, Scott 1988), northern Gulf of St. Lawrence cod (Templeman 1979, Moguedet 1994, Taggart et al. 1995), and southern Gulf of St. Lawrence cod (Jean 1964, Paloheimo and Kohler 1968).

While migratory patterns are well documented, very little is understood about the underlying triggering mechanism. Woodhead (1975) suggested that changing levels of circulating thyroid hormones in the bloodstream trigger migratory behaviour, since these hormones increase swimming speeds in both laboratory (Woodhead 1970, Castonguay and Cyr 1998) and field-released cod (Woodhead 1975). Similarly, gonadal hormones may also trigger migration because of the common linkage of migration and reproduction, and because testosterone enhances locomotor activity in teleosts (Hoar et al. 1952, 1955, Baggerman 1963, 1966). To date, however, the natural changes in the levels of endocrine hormones were not examined in relation to the extensive migrations of cod.

My goal was to determine whether thyroidal or reproductive hormones were correlated with the seasonal migrations of southern Gulf of St. Lawrence cod (Figure 3.1). Scientific surveys have identified the western Gulf as being the major summer ground for the stock (summarized by Swain 1999). A smaller component of the population summers in the eastern Gulf. In autumn, both the western and eastern components migrate to overwinter in the Cabot Strait along the southern edge of the Laurentian Channel (Jean 1964, Paloheimo and Kohler 1968, Halliday and Pinhorn 1982, Campana et al. 1999). The autumn fishery in the eastern Gulf is based largely on the western component migrating towards the Cabot Strait. In spring, the cod migrate back into the southern Gulf, thus completing the annual migration cycle. I monitored on a frequent basis the circulating levels of testosterone, estradiol-17β (E_2), L-thyroxine (T_4), 3,5,3'-triiodo-L-thyronine (T_3), and free (protein unbound) T_3 in relation to the onset of seasonal migrations.

MATERIALS AND METHODS

I investigated the timing of the autumn migration using sentinel survey data. Sentinel surveys consist of periodic sampling excursions on traditional fishing grounds following a scientific protocol. Two sentinel seiners carried out trips into the western Gulf from October to November in 1994, and from July to October in 1995, 1996 and 1997. During each trip, about 10 standardized sets were made at randomly chosen locations within the

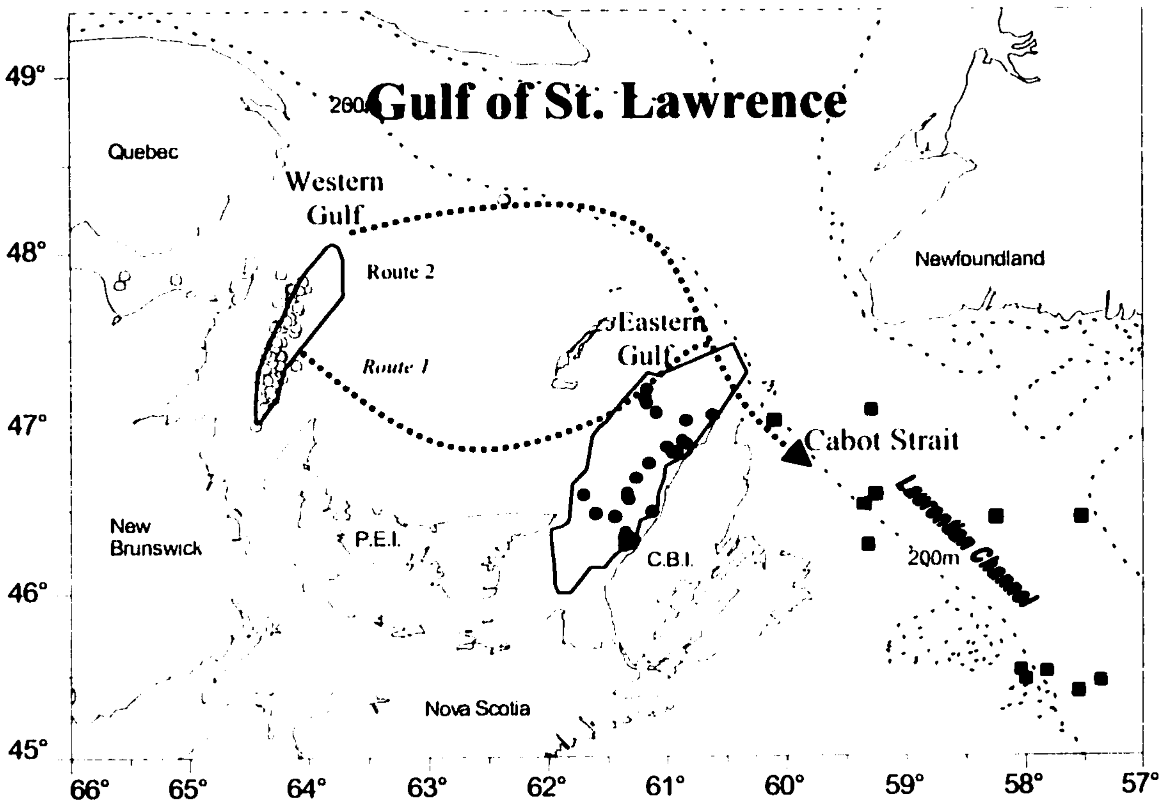

Figure 3.1. Capture locations of cod from 1994 to 1997, and the two most likely migration routes for the western Gulf component (Sinclair and Currie 1994, G. A. Chouinard, unpublished data) between the southern Gulf of St. Lawrence (summer) and the Cabot Strait (winter) grounds. Symbols indicate sampling stations in western Gulf area (○), eastern area (●) and Cabot Strait region (■). The ○ symbols outside the western Gulf represent sites visited in August and September 1994, prior to the beginning of sentinel surveys. Bold delimitation lines show zones inside which sentinel vessels operated.

western Gulf. I used the precipitous decline in catch rate as an indicator of the timing of departure. I also examined catch rates from sentinel vessel operations in the eastern Gulf (1996 to 1997) to identify arrival of fish from the western portion of the stock. Data from Sinclair and Currie (1994) were used to document spring migrations.

I collected samples in the Cabot Strait area during the winter and spring, and in the southern Gulf of St. Lawrence during the summer and autumn. Winter and spring samples were collected every second month in 1996 and 1997 whereas summer and autumn samples were taken on a near-weekly basis between 1994 and 1997. The number of fish collected during each trip ranged from 7 to 106 (mean = 30) and both sexes were generally well represented. The mean fork length of cod in samples varied from 46 to 60 cm, except for the August 1994 sample (mean FL = 67 cm).

Most samples were captured using mobile gear vessels that carried out 60 min sets. In October and November 1996, however, 43% of the eastern Gulf samples were collected using baited-longlines. The soaking time of longlines ranged from 4 to 24 h. No effects of gear-type on the concentrations of hormones were found (Mann-Whitney test, $p <$ 0.22) when comparing samples collected with mobile and longline gear in the same area on the same day. Blood samples were retrieved within about 20 minutes after the fish were boated. The aorta was cut and blood was allowed to flow directly into 7-ml Vacutainer vials. The vials were capped and refrigerated for 12 to 24 h. The serum fraction was then transferred into 2.5-ml cryovial tubes and stored at -72° C until analyzed. Cod were kept on ice and dissected within 48 h or frozen at -20° C and dissected after having been partially thawed. I determined the gonadosomatic index (I_G) as 100 × the wet weight of the gonads divided by the wet weight of the carcass (total body weight minus combined weight of liver, gonads, and stomach) (Schwalme and Chouinard 1999).

I measured T_4 and T_3 on 1,456 samples using a combined radioimmunoassay (RIA) technique (Omeljianuk et al. 1984) and the reagents employed by Cyr et al. (1998). Details of the procedures are also given in Comeau et al. (2000). I measured free T_3 levels on 638 samples using G-25 Sephadex columns (Eales and Shostak 1985). The free T_3 method is based on the following considerations. When serum is added to columns (pH 8.6) containing free ^{125}I-T_3, a complete equilibrium between ^{125}I-T_3 and serum proteins is rapidly achieved. [Mathematically, the ratio ^{125}I-T_3-protein/T_3-protein will become equal to the ratio free ^{125}I-T_3/free T_3.] When the serum is then washed through the Sephadex column, the radioactive protein-bound fraction will appear in the eluate whereas the radioactive free fraction will remain on the Sephadex. The free fraction is not quantified directly, but computed from the radioactivity measured in the eluted protein bound fraction.

Because most of the circulating T_3 is naturally bound to plasma proteins, sample dilution is necessary to detect small variations in the free T_3 fraction. For cod, linearity between the percentage of ^{125}I-T_3 bound to serum proteins (eluate) and the dilution of pooled serum samples was maintained down to a dilution factor of about 1:20 (serum:phosphate buffer). Therefore, all samples were diluted 1:10, which was well within the linearity limits of the dilution curve. In the first step of the procedure, 100 µl (*c.* 10,000 counts) of ^{125}I-T_3 tracer was pipetted onto G-25 Sephadex columns previously equilibrated in 0.1 NaOH. Free iodide contamination ($^{125}I^-$) was removed by introducing 3 ml of phosphate buffer into the columns. The contamination was measured and subtracted from the total counts added to the columns in step one. One ml of diluted serum was added to each column. Shortly after the serum had drained into the Sephadex, 3 ml of phosphate buffer was pipetted into the columns and the eluate containing the protein bound ^{125}I-T_3 was counted. Free T_3 percentage values were computed as in Eales and Shostak (1985), and then converted into concentration values using T_3 results from the combined T_4-T_3 RIA (i.e. total T_3) and the following formula:

$$\text{free } T_3 \,[\text{ng/ml}] = \text{total } T_3 \,[\text{ng/ml}] \times \text{free } T_3 \,[\%] \times 10^{-2}$$

Gonadal hormones were measured using commercial Coat-A-Count® RIA kits (Diagnostic Products Corporation, Los Angeles, CA). A total of 1,140 samples (436 males, 704 females) were processed in duplicate for the measurement of E_2 levels. A similar number of samples (576 males, 548 females) were analysed, also in duplicate, for testosterone levels.

For all RIA analyses, radioactivity was measured using a Beckman Gamma 5500 Counting System. The dilution of cod serum samples yielded hormone levels close to the predicted values, and the dilution curves of samples were parallel to the dilution curves of standards. Intra- and interassay coefficients of variation were less than 10%.

RESULTS

Because few samples were collected during the spring migration period, I decided to pool the 1996 and 1997 data in order to present the two sexes separately (Figure 3.2). In spring, the mean (1996 – 1997) I_G values were either increasing (females) or had already reached their maximum values (males) by March (Figure 3.2a). However, despite obvious gonadal development, testosterone levels remained relatively low in both sexes until June, at which time levels clearly peaked (Figure 3.2b). By contrast, E_2 in females reached and persisted at maximal levels from March to June (Figure 3.2c). Also, the E_2 : testosterone ratio clearly surpassed the 1.0 mark in March (Figure 3.2d), indicating that E_2 was more concentrated than testosterone. E_2 levels in males were extremely low (< 0.1 ng ml^{-1}) throughout the year and were not shown graphically. Thyroid hormone levels were either low or declining when cod migrated into the western Gulf during the spring of 1996 and 1997.

From 1994 to 1997, there was a prominent rise in sentinel catch rates in early October in the western Gulf, followed by sharp declines to null values in late October (Figure 3.3a). The timing of the autumn peaks ranged from October 2 to October 13 (mean = October 8), and thus was relatively constant from year to year (SE = 2 d). Major peaks and steep declines in catch rates were also detected in the eastern Gulf (Figure 3.3b). In this area, the peaks occurred in late October (mean = October 22, SE = 1 d), indicating the arrival of fish from the western Gulf.

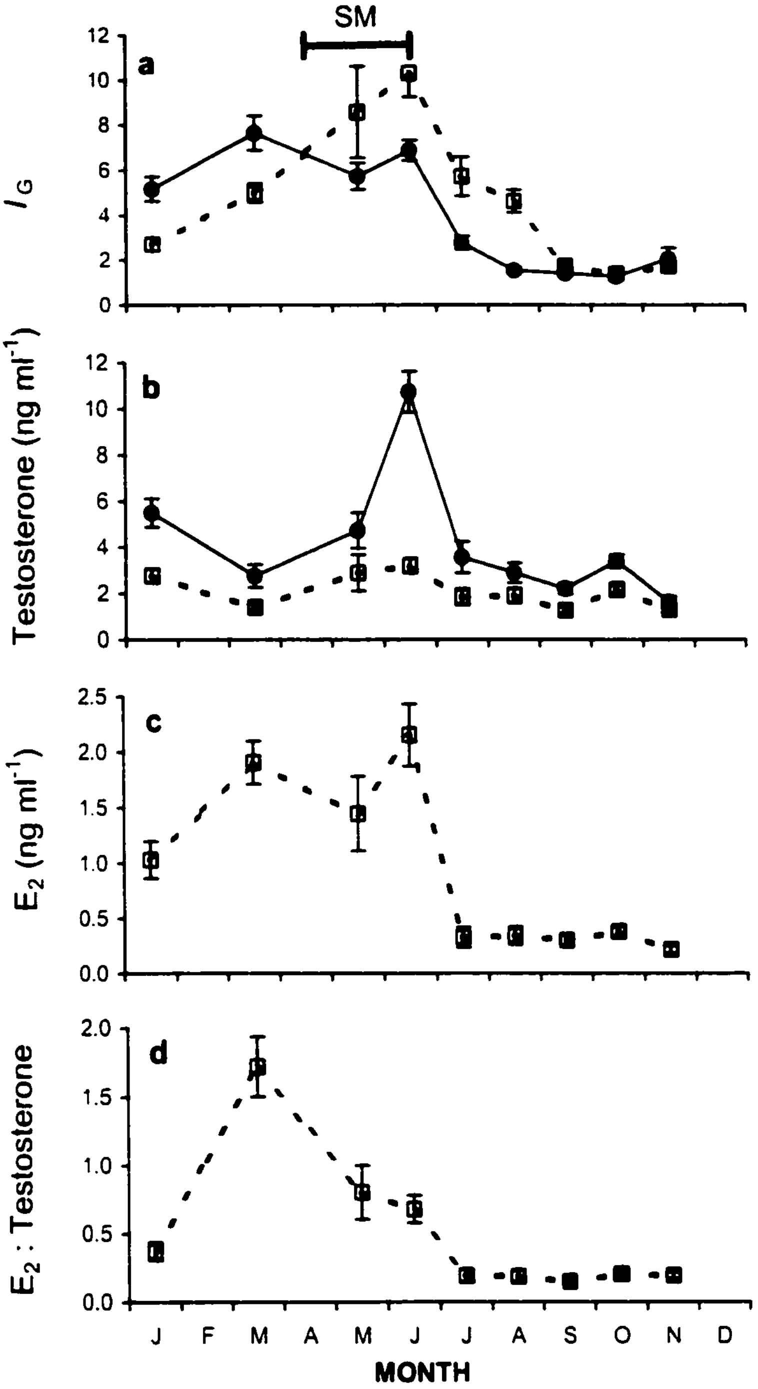

Figure 3.2. Seasonal changes in I_G, testosterone, E_2, and E_2 : testosterone ratio in male (●) and female (□) cod. Results from 1996 and 1997 were pooled to increase sample sizes. Data points represent monthly means (±SE). The 'SM' horizontal bar indicates the approximate timing and duration of the spring migration as inferred from Sinclair and Currie (1994) and July sentinel data.

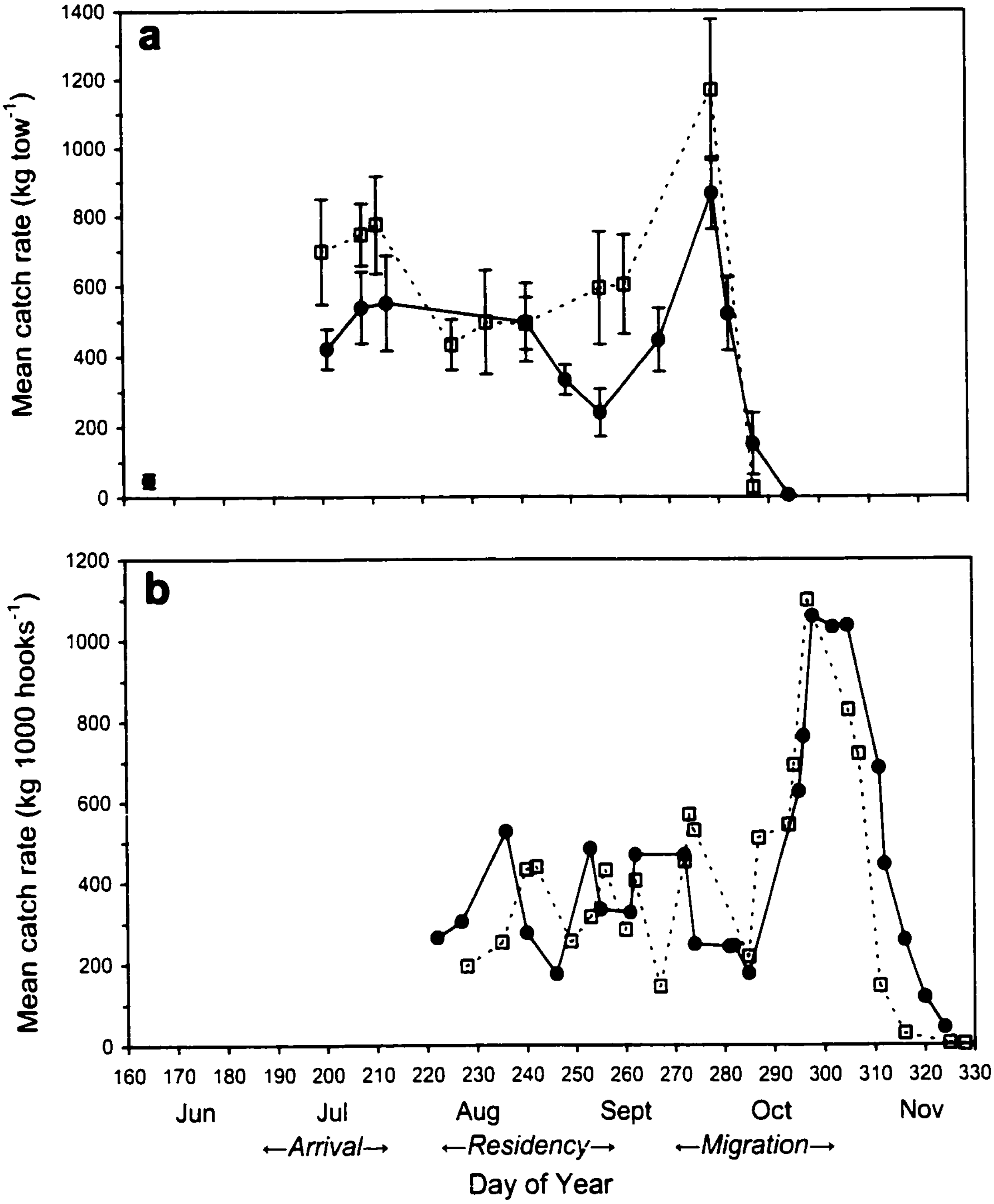

Figure 3.3. Changes in catch rates of cod during sentinel expeditions in 1996. In each graph, ● and □ symbols represent different survey vessels. In the western Gulf (graph a), data points are means (±SE) of 6 to 8 tows, except for June where only three tows were carried out during a separate research expedition. In the eastern Gulf (graph b), data points are catches per 1000 hooks (longline).

Testosterone levels usually remained low in males as they departed the western Gulf in 1995, 1996 and 1997 (Figure 3.4a). E_2 levels in females were also generally low at this time (Figure 3.4b), and no consistent pattern emerged from the free T_3 series (Figure 3.4c). However, serum T_4 levels peaked about 15 d before the maximum catch rates were recorded (Figure 3.5a, 3.5b). The mean timing of the T_4 peaks between 1994 and 1997 was September 23 (autumn equinox) with very little inter-annual variability (SE = 1 d). Consequently, I decided to group the thyroid data into three periods: summer residency, near autumn equinox, and autumn migration. Mean levels of thyroid hormones near the autumn equinox were significantly higher than those during the summer residency period by 2 to 3 ng ml^{-1} (T_4, Figure 3.6a) and 4 to 5 ng ml^{-1} (T_3, Figure 3.6b). In percentage terms, the equinoxial increases were 173% (T_4) and 26% (T_3) on mean. A comparatively small number of samples collected near the equinox in 1995 (16 compared to 29 - 37 fish in other years) might be responsible for the lack of statistical significance in that year.

While the levels of both thyroid hormones increased near the autumn equinox, T_4 showed greater year-to-year variability than T_3: the equinoxial T_4 upsurges differed between the investigated years ($p < 0.001$, Kruskal-Wallis test) whereas the equinoxial T_3 upsurges did not differ ($p < 0.21$, Kruskal-Wallis test). Also, for any given year, the T_4 levels recorded near the autumn equinox were highly variable from one individual to another, with the sample coefficient of variation (CV) ranging from 80 to 146%. In comparison, the sample CV for T_3 ranged from 26 to 34%. After the equinox, as cod quickly moved out of the western Gulf (Autumn Migration I in Figure 3.6a, 3.6b), the mean T_4 levels fell sharply but the mean T_3 levels remained elevated.

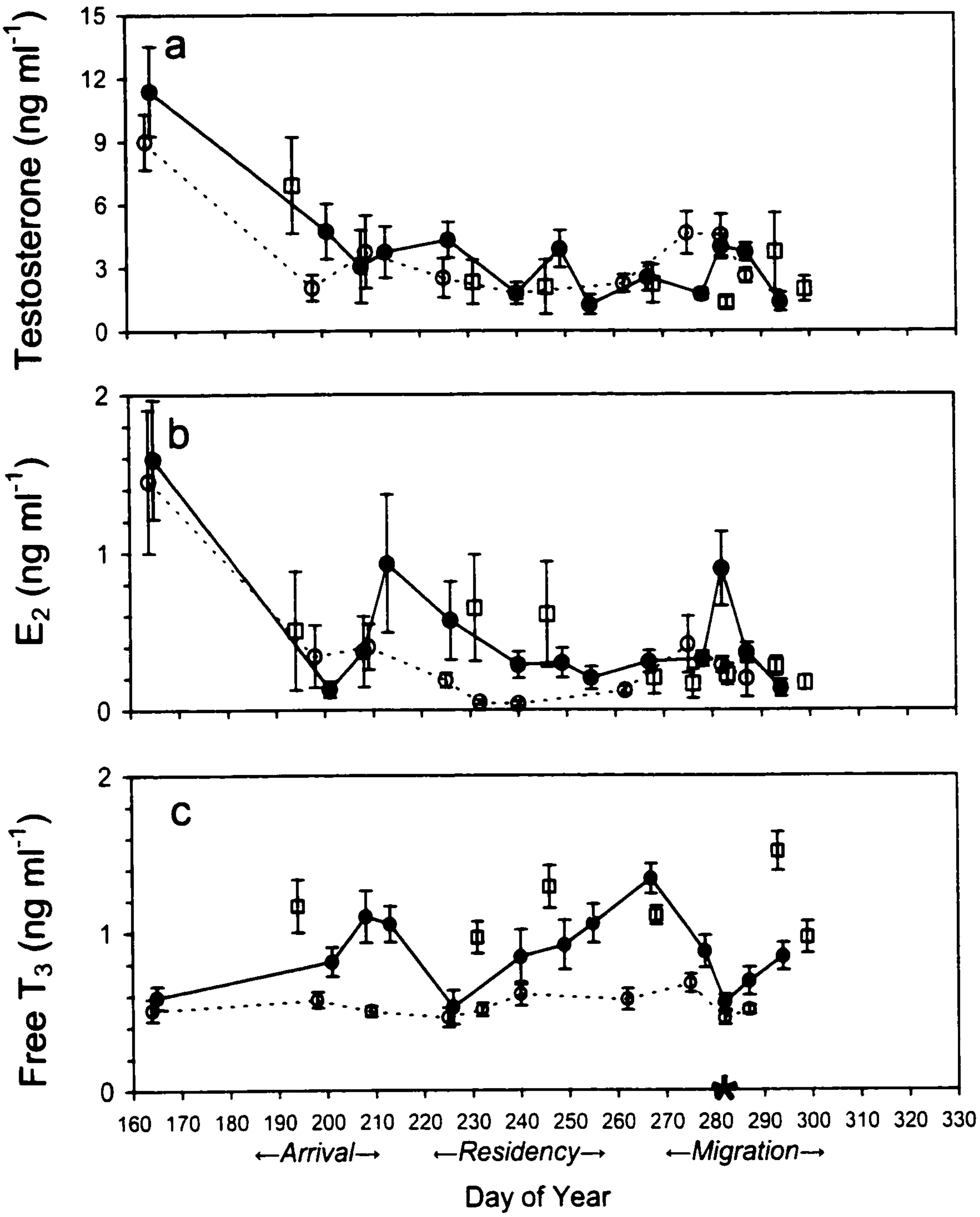

Figure 3.4. Summer and autumn testosterone (males), E_2 (females), and free T_3 (both sexes) levels in western Gulf cod in 1995 (□), 1996 (●), and 1997 (○). Data points represent expedition means (±SE). Data from 1994 have been omitted due to low sample sizes. The * symbol indicates the mean date (October 8, day 281) when the sentinel catch rates peaked before declining to null values.

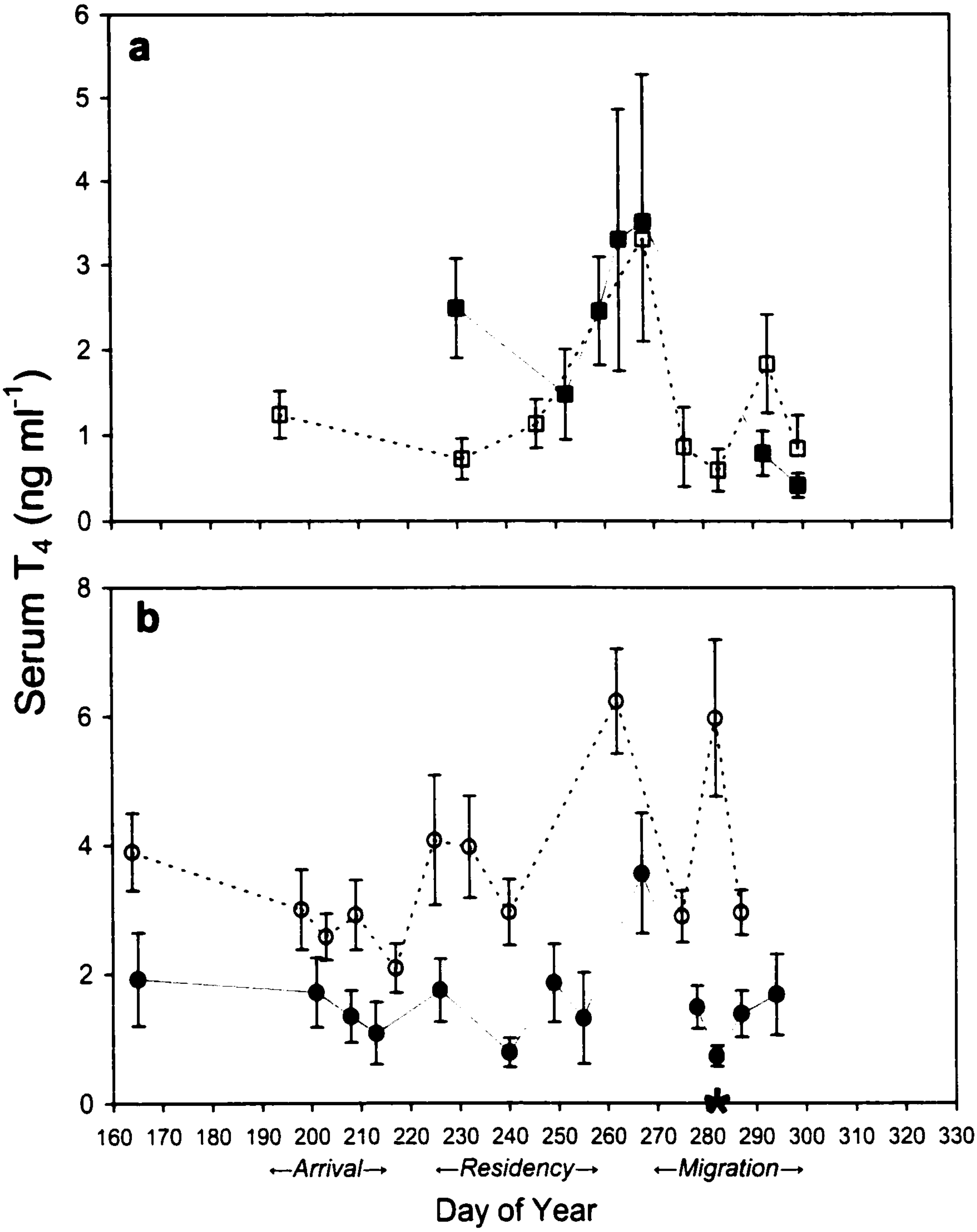

Figure 3.5. Summer and autumn T_4 levels in western Gulf cod in 1994 (■) and 1995 (□) (graph a), and in 1996 (●) and 1997 (○) (graph b). Data points represent expedition means (±SE). The * symbol indicates the mean date (October 8, day 281) when the sentinel catch rates peaked before declining to null values. September 23 corresponds to day 266.

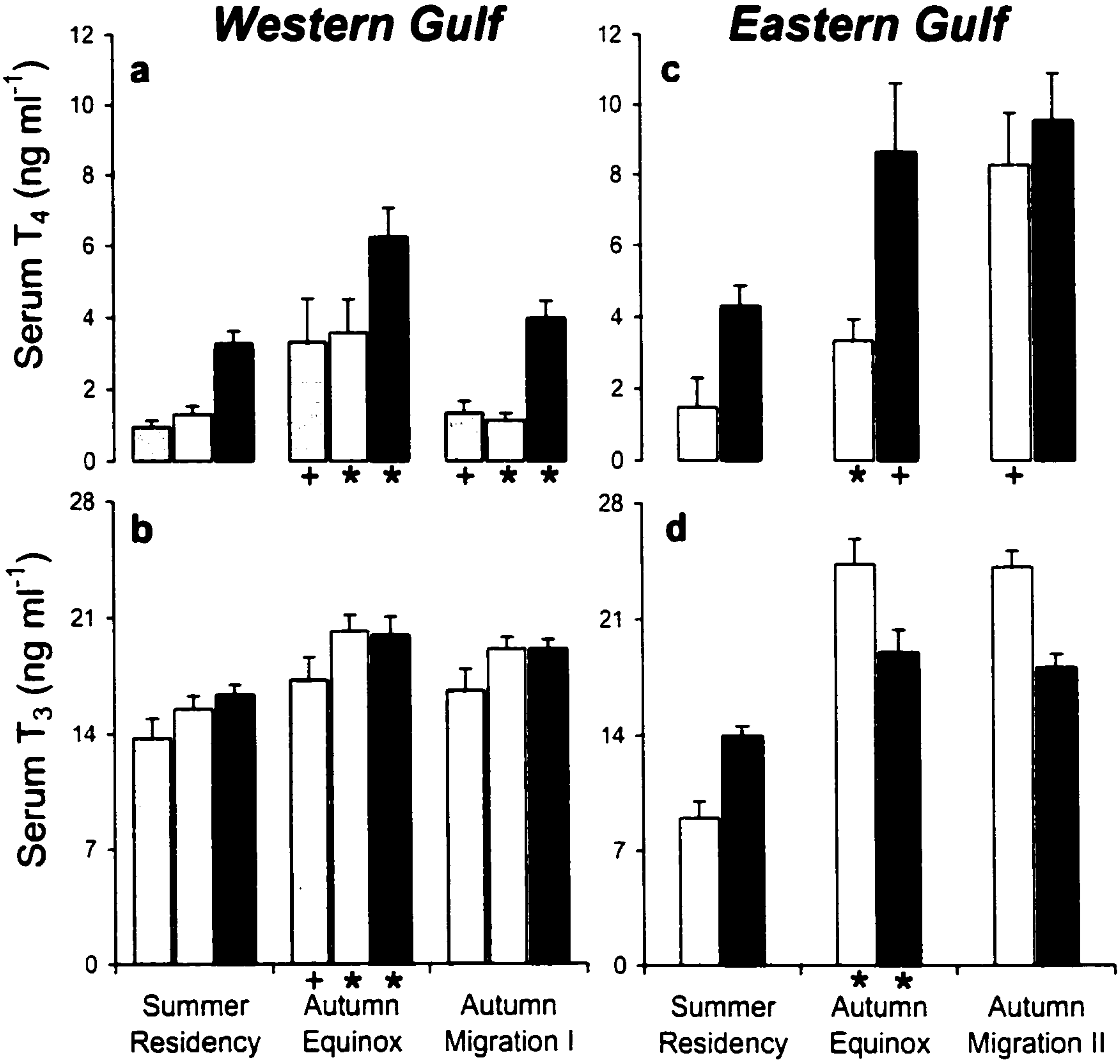

Figure 3.6. Mean (±SE) T_4 and T_3 levels during the summer residency, equinoxial, and migration periods in 1995 (grey bars), 1996 (open bars), and 1997 (black bars). Data from 1994 have been omitted due to low sample sizes. The 'Summer Residency' period includes measurements between June and the cruise closest to the autumn equinox; the 'Autumn Equinox' represents the cruise closest (within 7 d) to the autumn equinox; the 'Autumn Migration I' represents the start of the migration in the western Gulf; the 'Autumn Migration II' refers to the period when cod transited through the eastern Gulf. The * symbol indicates a significant ($p < 0.01$, Mann-Whitney two-sample test) difference between two consecutive periods; the + symbol refers to a significance level between 0.05 and 0.10 (Mann-Whitney two-sample test).

In the eastern Gulf, the concentrations of thyroid hormones also increased near the autumn equinox (Figure 3.6c, 3.6d). At this time, both T_4 and T_3 means were similar to the ones recorded in the western Gulf ($p > 0.05$, Mann-Whitney two sample test applied to 1996 and 1997 series separately). Also, as in the case of the western Gulf, the variation between individuals during the equinoxial period was greater for T_4 (CV = 124 to 135%) than for T_3 (CV = 40 to 49%). The levels of thyroid hormones remained elevated after the equinox (Autumn Migration II in Figure 3.6c, 3.6d), as western Gulf cod transited through the eastern Gulf.

DISCUSSION

A key assumption of this study is that the measured hormone levels were not significantly distorted by capture stress. It appears that capture stress has a minimal effect on the circulating levels of both T_4 and T_3 (see Comeau et al. 2000). Capture stress, however, is known to reduce the levels of gonadal hormones in various fishes (Pickering et al. 1987, Pankhurst and Dedual 1994, Jardine et al. 1996, Clearwater and Pankhurst 1997, Morehead 1998). Yet I note that testosterone levels in field-collected cod (this study) were generally higher than those reported for laboratory-held cod (Cyr et al. 1998). For cod, it may be that confinement stress (e.g., Pickering et al. 1987, Jardine et al. 1996, Clearwater and Pankhurst 1997, Morehead 1998, Haddy and Pankhurst 1999) has a greater impact on gonadal hormones than capture stress. Also, gonadal hormones in my study co-varied with the I_G in a manner consistent with other reports involving marine demersal fishes (Campbell et al. 1976, Wingfield et al. 1977, Johnson et al. 1991, Harmin et al. 1995, Cyr et al. 1998). Therefore, I suggest that the hormonal profiles in this study reflect natural changes.

I found that testosterone cycling in cod was not related to the onset of seasonal migrations. Circulating testosterone levels remained low in both males and females as they departed the western Gulf in autumn. It appeared that testosterone cycling in cod was more closely associated with the latter phases of gonadal development, and most likely with spermiation as in the case of various other fish (Liley and Stacey 1983), than with migratory behaviour. My observations concur with analyses of Barents Sea cod testis tissue that showed little histological signs of secretory activity at the beginning of a spawning migration (Woodhead and Woodhead 1965, Woodhead 1975). Thus, it seems unlikely that male androgens, or testosterone at least, are implicated in the mechanism cueing the seasonal migrations of cod.

Unlike testosterone, E_2 increased to maximal levels in March, and remained elevated until June when the spring migration was for the most part completed. Although this profile raises the possibility that E_2 was concerned with migratory behaviour, it remains unclear as to how exactly the hormone could have cued migration. Unlike androgenic steroids, E_2 has no documented influence on swimming activity. One synthetic oestrogen, stilboestrol, increased swimming speeds in goldfish, but its effects were always less pronounced than the ones resulting from treatments with male sex hormones (Hoar et al. 1955). I found E_2 levels negligible in the male, which implies that males do not undergo endocrinological changes favouring migration, but rather that they follow females on their migratory journey. In addition, the annual rise in E_2 levels might not be synchronized with spring migratory behaviour in other cod populations. For example, unlike southern Gulf of St. Lawrence cod, Barents Sea cod and northern cod undertake their spring migrations as recently spent fish (Lear and Green 1984, Rose 1993, Wroblewski et al. 1995, Ponomarenko 1996). While endocrine hormones were not reported for these populations, E_2 in externally fertilizing teleosts usually reaches maximal concentrations during late ovarian development, declines substantially at the time of spawning, and remains low for a considerable amount of time after spawning (Fostier et al. 1983, Liley and Stacey 1983, Methven et al. 1992, Harmin et al. 1995, this study). It seems reasonable to assume that E_2 levels are very low in (spent) Barents Sea cod and northern cod when they engage in spring migration; consequently, a functional link between E_2 and migratory behaviour is very doubtful for cod.

In autumn, T_4 levels in western Gulf cod invariably peaked close to the autumn equinox (September 23 1994 to 1997), with the mean timing of the T_4 peaks being September 23 (SE = 1 d). Equinoxial T_4 surges were also detected in fish from the eastern Gulf, suggesting that the thyroidal event was widespread. These results imply relative daylight/night time hours as a key signal for enhancing thyroid function in autumn. Woodhead and Woodhead (1965) reached a similar conclusion for Barents Sea cod based on thyroid follicular cell height.

My data also show a post-equinoxial decrease of T_4 levels in western Gulf cod. It seems unlikely that the lowering of T_4 was caused by reduced T_4 release from the thyroid, as thyroid function in cod increases through the autumn (Woodhead 1959, Comeau et al. 2000). Alternatively, it is possible that the post-equinoxial lowering of T_4 was brought about by differential migration rates. More precisely, high T_4 individuals might have moved out of the Gulf faster than their low T_4 counterparts, leading us to sample an increased proportion of low T_4 individuals in the western Gulf as the autumn migration progressed eastward. I base this interpretation on previous work showing that T_4 increased swimming speeds in laboratory cod (Woodhead 1970, Castonguay and Cyr 1998) and ground speeds in wild cod (Woodhead 1975). In the open sea experiment, cod implanted subdermally with a T_4 pellet reached the overwintering area significantly faster than the control individuals (Woodhead 1975). The peaks in sentinel catch rates in early October (mean = October 8 1994 to 1997), and the subsequent declines to null values within days, are also consistent with the idea that that migratory restlessness is initiated close to the equinox. It is likely that the peak catch rates reflected a migration well under way, and not the onset of migratory behaviour *per se*. The reason is that adult cod were probably dispersed throughout the summer feeding area when they sensed the appropriate cue(s) to start migrating, and that a certain amount of time was required before they could join and form large aggregations (see Rose 1993) that ultimately influenced catch data. In brief, the post-equinoxial lowering of T_4 may be viewed as an endocrine signature marking the onset of migration (September 23).

Woodhead (1959, 1975) suggested that enhanced thyroidal status might motivate Barents Sea cod to turn and swim against the prevailing Norwegian current. For southern Gulf of St. Lawrence cod, however, thyroid hormone levels during the upstream migration (spring) were either equal or lower than those recorded during the downstream migration (autumn). I also found no evidence that cod migrations were cued to a specific thyroid activity level. As cod initiated migration in the western Gulf, T_4 levels were highly variable amongst individuals, with sample CVs ranging from 80 to 146%. It could be that individuals have different behavioural thresholds to T_4. This however seems unlikely as the mean T_4 levels at the onset of migration (equinox) differed significantly across years, suggesting that the mean endocrinological threshold for reactivity would somehow vary from one year to the next. Nevertheless, my data showed that migratory behaviour was triggered after the mean concentration of circulating T_4 had reached 2 to 3 ng ml^{-1} above the summer residency baseline. High T_4 secretion at the autumn equinox may ensure migratory readiness by enhancing both sensory physiology (Hoar et al. 1955) and motor capacity (Woodhead 1970, Castonguay and Cyr 1998). The equinoxial T_4 event coupled to the year-to-year consistency in migration timing identifies photoperiod as a likely external cue.

Mean T_3 levels increased by 4 to 5 ng ml^{-1} at the onset of migration (equinox) and remained elevated throughout the migration. T_3 might have facilitated the autumn migration by having a generalized excitatory effect upon metabolism because it binds to (putative) nuclear receptor sites with greater affinity than T_4 (Eales and Brown 1993, Cyr and Eales 1996). However, free T_3, which is the form that can potentially interact with nuclear receptor sites, showed no reoccurring pattern in relation to migration. It is possible that the lack of free T_3 pattern was attributed to the hormone being metabolized as quickly as it was being released from plasma binding proteins.

In summary, major endocrine changes occurred in the Atlantic cod as it migrated seasonally. Serum E_2 levels in females peaked in early spring and declined only after the cod had reached its spawning grounds. In contrast, the course of thyroid hormones was concurrent with the autumn migration, with T_4 and T_3 levels increasing shortly before the onset of the autumn migration was apparent from catch rate data. I suggest that the changes in E_2 levels were functionally tied with egg development and not with spring migratory behaviour, and that thyroid hormones exerted physiological effects favouring the onset of the autumn migration. The year-to-year consistency in the timing of migration pointed to photoperiod as a likely external trigger. The indications of possible links between photoperiod, thyroid hormones, and migratory behaviour should be followed up by experimental studies.

CHAPTER FOUR

Correlations between thyroidal and reproductive endocrine status in wild Atlantic cod

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ABSTRACT

Serum levels of thyroid and gonadal hormones were monitored over two complete reproductive cycles in Atlantic cod (*Gadus morhua*) in order to identify potential interactions between the thyroidal and reproductive systems. I found few indications of interplay between the two endocrine systems in males. However, levels of total (protein bound and unbound) L-thyroxine (T_4) and total 3,5,3'-triiodo-L-thyronine (T_3) increased substantially during ovarian recrudescence in autumn. Thereafter, total T_3 levels declined after the ovaries reached a certain size (gonadosomatic index *c*. 3.0) and after estradiol-17β (E_2) rose above 1.0 ng ml^{-1}. Also, a free (protein unbound) T_3 index in females became inversely correlated with rising levels of both E_2 and testosterone. I suggest that the thyroidal system exerted a stimulatory effect on early ovarian development, but that gonadal hormones curtailed the peripheral thyroid status once a commitment to reproduction had been made. The biological implications of such interactions are discussed.

INTRODUCTION

In salmonid and freshwater teleosts, there is mounting evidence that complex interactions between the thyroidal and reproductive endocrine systems provide some control over competing somatic and gonadal growth processes (see Dickhoff et al. 1989, Cyr and Eales 1996). In marine fish, on the other hand, very little is known about the interplay between the two endocrine systems. For instance, Woodhead (1959, 1975) argued that major seasonal changes in thyroidal activity in the Barents Sea cod *Gadus morhua* are more closely related to migratory behaviour than to reproductive function. Since then the course of thyroid hormones (TH) over at least one complete reproductive cycle has been documented for plaice *Pleuronectes platessa* (Osborn and Simpson 1978), winter flounder *Pseudopleuronectes americanus* (Eales and Fletcher 1982), and more recently cod (Comeau et al. 2000). In these investigations, no significant correlations between thyroidal and reproductive status were found (plaice, flounder) or reported (cod).

Possible reasons for the difficulty in detecting links between the two endocrine systems include the criteria used for the assessment of endocrine status. The thyroid histological indices used in the study of Barents Sea cod (Woodhead 1959, 1975) are now considered unreliable for detecting short-term changes in the circulating levels of thyroid hormones (Eales and Brown 1993, Cyr and Eales 1996). In marine pleuronectiformes, TH levels were compared with gonadal condition indices (Osborn and Simpson 1978, Eales and Fletcher 1982), which were probably not proportional to gonadal hormone levels during certain phases of the reproductive cycle (e.g., see Pankhurst and Conroy 1987, Pankhurst and Kime 1991, Methven et al. 1992, Kjesbu et al. 1996). Moreover, because only total (protein bound and unbound) TH levels were measured in pleuronectiformes (Osborn and Simpson 1978, Eales and Fletcher 1982), certain interactions between the reproductive and peripheral thyroid systems may have gone undetected. Cyr and Eales (1989, 1992), for example, have shown that E_2 injection reduces the proportion of TH circulating in the free form in rainbow trout *Oncorhynchus mykiss* and *Oncorhynchus mykiss gairdneri*.

In this paper, my main objective was to identify potential interactions between the thyroidal and reproductive system in cod. I closely monitored the natural course of endocrine hormones, including E_2 and free 3,5,3'-triiodo-L-thyronine (T_3), over two complete reproductive cycles. My results led to the conclusion that interactions between the two endocrine systems in this marine teleost are plausible, particularly in females. I discuss the biological implications arising from these results.

MATERIALS AND METHODS

Samples attributes

Sexually matured Atlantic cod were sampled from the southern Gulf of St. Lawrence population between July 1995 and November 1997. The number of fish collected each month ranged from 13 to 224 (average = 91) and the two sexes were generally well represented. The mean fork length of cod in samples varied from 47 to 57 cm (average = 52 cm, SE = 0.5 cm). Most samples were captured using mobile gear vessels that carried out 60 min sets. In October and November 1996, however, 43% of the samples were collected using baited-longlines. The soaking time of longlines ranged from 4 to 24 h. No effects of gear-type on the concentrations of hormones were found (Mann-Whitney test, $p < 0.22$) when comparing samples collected with mobile and longline gear in the same area on the same day.

Blood samples were retrieved within about 20 minutes after the fish were boated. The aorta was cut and blood was allowed to flow directly into 7-ml Vacutainer vials. The vials were capped and refrigerated for 12 to 24 h. The serum fraction was then transferred into 2.5-ml cryovial tubes and stored at -72° C until analysed. Cod were kept on ice and dissected within 48 h or frozen at -20° C and dissected after having been partially thawed. I determined the gonadosomatic index (I_G) as 100 × the wet weight of the gonads divided by the wet weight of the carcass (total body weight minus combined weight of liver, gonads, and stomach) (Schwalme and Chouinard 1999).

Hormone measurements

I measured total L-thyroxine (T_4) and total T_3 on 1,665 serum samples using a combined radioimmunoassay (RIA) technique (Omeljianuk et al. 1984) and the reagents employed by Cyr et al. (1998). Details of the procedures are also given in Comeau et al. (2000). Of the 1,665 samples, 856 contained a sufficient amount of serum to determine the fraction of T_3 that was freed from carrier proteins. A free T_3 index, representing the percentage of serum T_3 in the free form, was measured on G-25 Quik-Sep columns (Isolab Inc., Akron, OH) according to the procedure of Eales and Shostak (1985) adapted for cod serum

(Comeau et al. *in press*). The free T_3 index was converted into concentration values using total T_3 results from the combined T_4-T_3 RIA and the following formula:

$$\text{free } T_3\ [\text{ng ml}^{-1}] = \text{total } T_3\ [\text{ng ml}^{-1}] \times \text{free } T_3 \text{ index } [\%] \times 10^{-2}$$

Gonadal hormones were measured using commercial Coat-A-Count® RIA kits (Diagnostic Products Corporation, Los Angeles, CA). A total of 1,115 samples (429 males, 686 females) were processed for the measurement of E_2 levels. A similar number of samples (569 males, 530 females) were analysed for testosterone levels.

Radioactivity was measured on duplicate samples using a Beckman Gamma 5500 Counting System. Except for free T_3 (not applicable), the dilution of serum samples yielded hormone levels close to the predicted values, and the dilution curves of samples were parallel to the dilution curves of standards. Intra- and interassay coefficients of variation were less than 10%.

Statistics

Total thyroid hormones

Monthly means of total TH and the I_G were plotted against time. To examine the thyroidal profiles as a function of reproductive status, I ranked the reproductive variable (I_G, E_2, or testosterone) based on percentile groups: 25 groups were created, with each group containing approximately 36 cases (individuals). Arithmetic means of reproductive and thyroidal measurements were computed for individual groups; the means were plotted against each other and visually inspected for trends.

Free T_3

Simple and multiple linear regression analyses were used to investigate the relationships between the free T_3 index and three explanatory variables (E_2, testosterone, total T_3). The analyses were based on individual fish and were restricted to narrow phases of the reproductive cycle, each extending over a three-month period. For females, the free T_3 index was $\log_{10}$ transformed to ensure linearity; for males, the total T_3 variable was $\log_{10}$ transformed. In multiple regression analysis, I tested the following models for females and males, respectively:

$$\log_{10} \text{free } T_3 \text{ index} = \beta_0 + \beta_1 \cdot E_2 + \beta_2 \cdot \text{testosterone} + \beta_3 \cdot \text{total } T_3$$

$$\text{free } T_3 \text{ index} = \beta_0 + \beta_1 \cdot E_2 + \beta_2 \cdot \text{testosterone} + \beta_3 \cdot \log_{10} \text{total } T_3$$

where β_0 represents the intercept and $\beta_{1\rightarrow3}$ are the slopes. The entry and removal of independent variables from the models was based on a stepwise selection approach; the *P*-to-enter and *P*-to-remove values were set at 0.05 and 0.10, respectively. Residual plots and the Durbin-Watson test indicated that the error terms met the assumptions of linear regression analysis.

RESULTS

Total thyroid hormones

Total TH levels generally increased during gonadal recrudescence in autumn (Figure 4.1). However, the magnitude of the T_4 upsurge was much higher—by 85% on average—in females than in males ($p < 0.01$, t-test for paired comparisons applied to November – March monthly means). Moreover, the timing of the T_4 upsurge seemed more closely associated with early ovarian growth (I_G *c.* 1.0 – 3.0, Figure 4.2a) than with early testes growth (Figure 4.2b). Similarly, total T_3 levels were higher in females than males during the same period ($p < 0.05$, t-test for paired comparisons applied to October – March monthly means), and rising total T_3 levels co-varied with early ovarian development (Figure 4.2a).

Both total T_4 and total T_3 levels started declining abruptly during late ovarian development (I_G *c.* 3.0, Figure 4.2a). In males, total T_3 levels declined (slightly) when the testes reached a certain size (I_G *c.* 2.0, Figure 4.2b). Testosterone and E_2 levels were elevated during late gonadal development (Figure 4.2c, d). However, total T_4 levels presented no trend in relation to rising gonadal hormone levels (Figure 4.2e, f). Only total T_3 levels in females clearly started falling as E_2 levels rose above 1.0 ng ml^{-1} (Figure 4.2e).

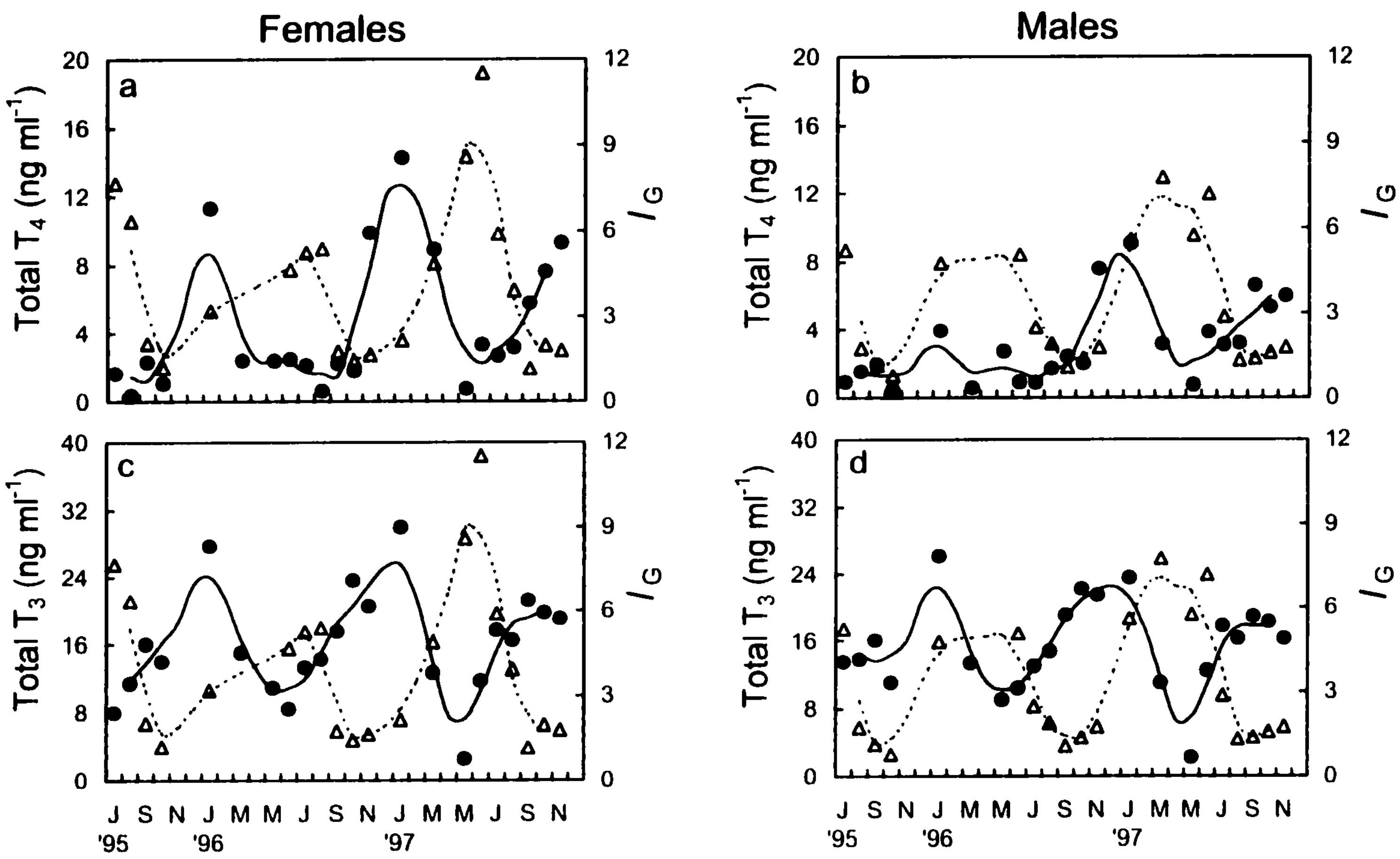

Figure 4.1. Seasonal changes of total TH and I_G shown separately for males and females. ●, Monthly means of either total T_4 [(a), (b)] or total T_3 [(b), (c)]; Δ, monthly means of I_G. Trend lines show three-point centred moving averages for hormones (—) and I_G (---) series. Missing months were replaced with computed values (linear interpolation of two neighbouring values) before running the three-point centred average function.

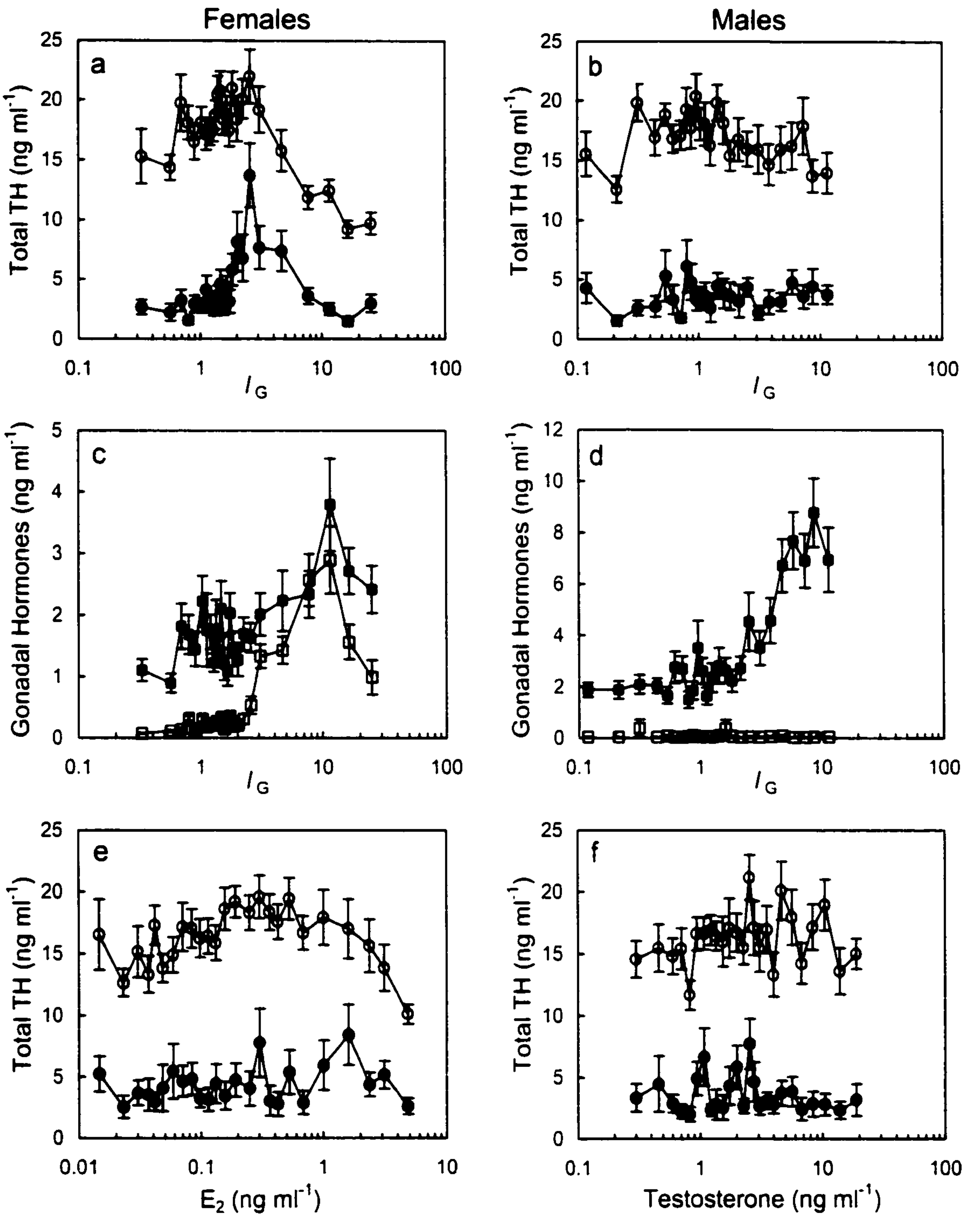

Figure 4.2. Interrelationships between total TH, I_G, and gonadal hormones plotted separately for males and females. Data points represent means from 25 percentile groups categorized based on variable x. Data collected throughout the year were included in the analysis. ●, Total T_4; ○, total T_3; □, E_2; ■, testosterone. Error bars show S.E.

Free T_3

The free T_3 index presented two major seasonal shifts (Figure 4.3a): it declined significantly in females in winter (autumn *v.* winter, $p < 0.01$, Mann-Whitney test), and increased in both sexes in spring (winter *v.* spring, $p < 0.001$, Mann-Whitney test). These seasonal changes are noteworthy because they rendered the absolute free T_3 levels less variable across seasons (Figure 4.3b). For instance, mean free T_3 levels in females could have risen to 1.8 ng ml^{-1} (SE = 0.2) in winter had the free T_3 index been fixed to the autumnal values; in reality, mean free T_3 levels in females peaked at 1.3 ng ml^{-1} (SE = 0.2) in winter. By shifting its course again in spring, the free T_3 index upheld free T_3 levels close to 0.6 ng ml^{-1} (SE = 0.1) in both sexes. Without this second shift, free T_3 levels might have declined to values near 0.36 ng ml^{-1} (SE = 0.04).

I found negative correlations between gonadal hormones and the free T_3 index in females in winter (Figure 4.4). E_2 and testosterone respectively explained 51% ($p < 0.001$) and 60% ($p < 0.001$) of the variability associated with the free T_3 index between the months of November and January. Testosterone was retained as the strongest predictor in a multiple regression model that explained 68% ($p < 0.001$) of the variability in free T_3 (Table 4.1). In spring, however, total T_3 became the best predictor of changes in free T_3 (Table 4.1; Figure 4.5). According to the models, the tendency to free T_3 from plasma proteins in spring was due in large part to low T_3 availability. Also, the relationships were significant only for cases (individuals) with total T_3 levels below 35 ng ml^{-1} (cases ≥ 35 ng ml^{-1} not shown in Figure 4.5), indicating a possible threshold for triggering the release of T_3 from binding proteins.

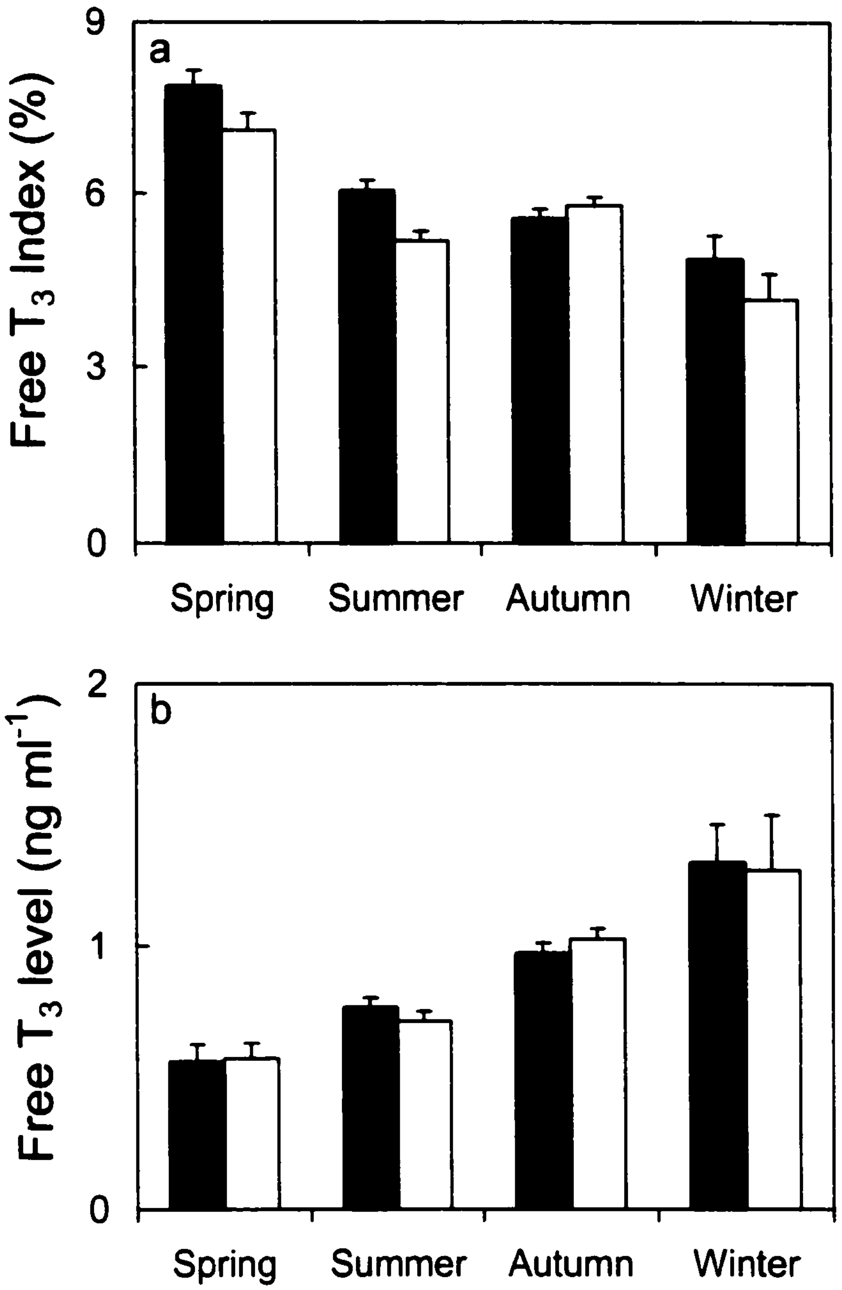

Figure 4.3. Seasonal averages of the free T_3 index (a) and free T_3 levels (b) in males (filled bars) and females (open bars). Error bars show S.E.

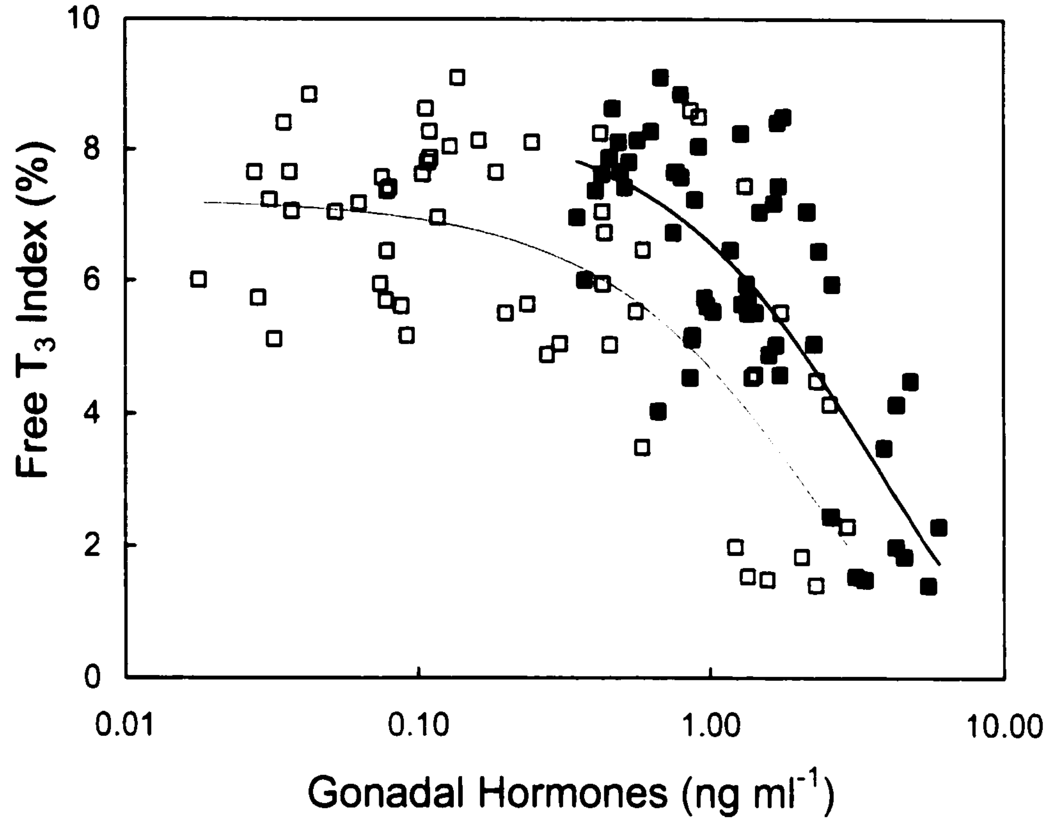

Figure 4.4. Relationships between the $\log_{10}$ free T_3 index and E_2 (□, $r^2 = 0.51$, $p < 0.001$) in females, and also between the $\log_{10}$ free T_3 index and testosterone (■, $r^2 = 0.60$, $p < 0.001$) in females. Data points represent individual fish sampled between November and January (1996 and 1997).

Table 4.1. Results of stepwise multiple regression analyses with the free T_3 index as the dependent variable. The t statistics provides an indication of the relative importance of each parameter; the *P* and Probability values indicate the significance of individual parameter and of the overall regression, respectively.

	E_2		Testosterone		Total T_3		Final Model	
Period	t	*P*	t	*P*	t	*P*	r^2	Probability
Nov – Jan (♀)	-1.63	0.11†	-8.94	<0.001	-2.82	<0.01	0.68	<0.001
Jan – Mar (♀)	0.37	0.72†	-3.43	<0.01	-4.14	<0.001	0.58	<0.001
(♂)	0.88	0.39†	-0.36	0.73†	-6.71	<0.001	0.58	<0.001

† parameter excluded from model

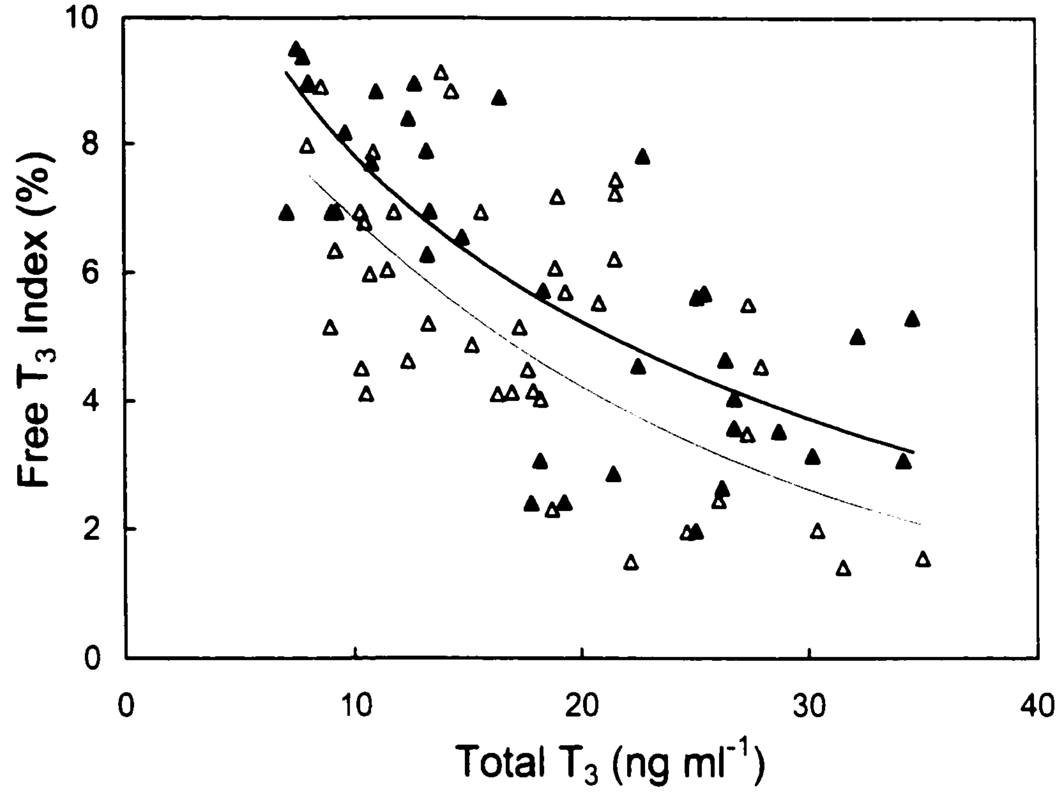

Figure 4.5. Relationships between the $\log_{10}$ free T_3 index and total T_3 levels in females (Δ, $r^2 = 0.45$, $p < 0.001$), and also between the free T_3 index and $\log_{10}$ total T_3 levels in males (▲, $r^2 = 0.58$, $p < 0.001$). Data points represent individual fish sampled between January and March (1996 and 1997).

DISCUSSION

Effects of thyroid hormones on reproductive function

Woodhead (1959, 1975) suggested that enhanced thyroidal activity in cod is functionally related to migratory behaviour. In the present study, I report that TH may also aid in the initiation of new reproductive cycles. I found appreciable total T_3 levels during testicular recrudescence, consistent with enhanced T_3 concentrations in the testes of chum salmon *Oncorhynchus keta* (Tagawa et al. 1994), and also with the presence of nuclear T_3 receptors in the testes of freshwater perch *Anabas testudineus* (Jana and Bhattachcharya 1993). The thyroidal upsurge in relation to ovarian recrudescence was particularly remarkable and clearly involved both T_3 and T_4. In freshwater and salmonid species, it was shown that TH act in synergy with gonadotrophins to stimulate early ovarian follicle growth (Cyr and Eales 1988, 1996); also, it is suspected that T_4 enhances vitellogenin uptake from plasma into the eggs (Shibata et al. 1993, Cyr and Eales 1996). Hence, I cannot reject the hypothesis that TH in cod are involved in early oocyte maturation.

Effects of gonadal hormones on thyroidal function

Some studies on salmonids suggested that rising E_2 levels during the later stages of ovarian development exert a negative effect on the thyroid gland, thereby reducing T_4 levels in circulation (Leatherland 1985, Yamada et al. 1993). Despite a close association between late ovarian growth ($I_G > 3.0$) and falling total T_4 levels in cod, I found no inverse correlation between E_2 and T_4. This result underscores the fact that gonadal condition can be misleading in terms of identifying relationships between gonadal hormones and thyroidal function. Late ovarian growth and falling total T_4 levels in cod are likely two independent processes governed by the same environmental variable, such as photoperiod (see Lam 1983, Brown and Stetson 1985, Grau et al. 1985, Brown 1988, Sumpter 1990, Comeau et al. 2000). Therefore, I suggest that gonadal hormones such as E_2 do not negatively affect the thyroid gland in cod.

On the other hand, my data are in agreement with the premise that E_2 suppresses the peripheral thyroid status, namely T_3 production rates (Leatherland 1985, Cyr et al. 1988, Flett and Leatherland 1989, Yamada et al. 1993) and the free T_3 index (Cyr and Eales 1989). I also found that testosterone might have contributed to the lowering of the free T_3 index. Both steroids were present in appreciable concentrations in females, and their combined effect provides a reasonable explanation for the free T_3 index declining significantly in females in winter. Falling (< 35 ng ml^{-1}) total T_3 levels, however, seemed largely responsible for the elevation of the free T_3 index in spring. This finding is consistent with the observation that arctic charr *Salvelinus alpinus* with low total T_3 levels contained relatively few T_3 binding proteins (Eales and Shostak 1985). In cod, as in many other teleosts, the liver manufactures the bulk of the circulating T_3 (Eales and Brown 1993, Cyr et al. 1998). Poor feeding conditions on winter grounds (Schwalme and Chouinard 1999) likely depressed hepatic T_3 production rates in southern Gulf cod (Comeau et al. 2000), perhaps triggering in turn a reduction in the number of T_3 binding proteins. I conclude that gonadal hormones are probably not the only factors influencing the free T_3 index in wild cod, especially at times when T_3 production rates fall below a certain threshold.

Biological implications

In cod, the release of T_4 by the thyroid is activated at the autumn equinox (Comeau et al. in press). Therefore, endocrine stimulation of ovarian recrudescence may be synchronized in several cod populations throughout the northern hemisphere. To date, evidence of ovarian cycles being initiated near the autumnal equinox is available for southern Gulf of St. Lawrence cod (Schwalme and Chouinard 1999), northern Gulf of St. Lawrence cod (Lambert and Dutil 1997b), Arctic cod (Hop et al. 1995), Barents Sea cod (Woodhead and Woodhead 1965, Ponomarenko 1996), and Pacific cod (Smith et al. 1990).

Once ovarian growth is under way, continued interactions between the two endocrine systems may allow some control over competing somatic and gonadal growth processes. More precisely, it is known that the free T_3 fraction is metabolically potent since it can readily move into target cells and interact with nuclear receptor sites (Eales and Brown 1993). It is also known that T_3 is more effective than T_4 in promoting fish growth (Higgs et al. 1979). Accordingly, in the female cod, the curtailment of free T_3 levels in winter likely restricts the metabolism and somatic growth rates, and in doing so may allow the metabolic reserves to be directed towards continued oocyte development (Cyr and Eales 1996). During the spring period, there are probably some adaptive advantages in upholding free T_3 levels above a certain threshold. This second peripheral endocrine adjustment, which was unexpected and poorly correlated with reproductive status, may be necessary for sustaining cell metabolism during a lengthy (500 km – Hanson 1996) spawning migration in spring.

In summary, I found that interactions between the thyroidal and reproductive systems in cod were plausible for certain phases of the reproductive cycle, particularly in females. It is suggested that TH contributed to the stimulation of ovarian recrudescence, and that rising levels of E_2 and testosterone, both present in females, exerted a negative effect on the peripheral thyroid status. Despite major differences in reproductive strategies, similar interactions were reported for salmonids (Cyr and Eales 1996). Thus, it appears that the interactions between the two endocrine systems are important regulators of basic physiological processes.

CHAPTER FIVE

Modifying thyroidal status in Atlantic cod by osmotic pump delivery of thyroid and anti-thyroid agents

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ABSTRACT

The objective of this study was to determine whether osmotic pump delivery of 3,5,3'-triiodo-L-thyronine (T_3), thyroid inhibitors (potassium iodide, methimazole, thiourea), or gonadal hormones (estradiol-17β, testosterone) could modify the thyroidal status of wild Atlantic cod *Gadus morhua*. Osmotic pumps implanted intraperitoneally delivered on average 3% (SE = 0.5%) of their initial volume per day under the experimental conditions (depth 25 m, 5.2 to 7.6°C). The administration of T_3 significantly increased serum levels of both T_3 and L-thyroxine (T_4). Treatment with either methimazole or thiourea significantly depressed serum T_3 levels to about 45% of control values, whereas treatment with testosterone significantly reduced T_4 levels by about 65%. I conclude that the delivery of thyroid or anti-thyroid agents by osmotic pumps is an effective means for creating sustained (1 month) experimental hyper- or hypothyroidism in wild cod.

INTRODUCTION

There is growing evidence that marine demersal species undergo prominent annual cycles in thyroid activity (Osborn and Simpson 1978, Eales and Fletcher 1982, Comeau et al. 2000). While the functional role of these cycles has yet to be determined, it has long been suspected that they are involved in the mechanism triggering seasonal migrations (Woodhead 1959, 1975, Osborn and Simpson 1978, Lear and Green 1984). In keeping with this hypothesis, Comeau et al. (in press) reported that blood levels of L-thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) in wild Atlantic cod *Gadus morhua* peaked only days before the onset of the overwintering migration. Woodhead (1970) and Castonguay and Cyr (1998) in turn noted enhanced locomotory activity in captive cod injected with T_4. However, it is not known whether an increase in locomotory activity should be interpreted as a motivation to engage in seasonal migration.

An alternate and straightforward approach for verifying the postulated link between the thyroid and migration would consist of tracking thyroid-manipulated subjects in their natural environment. Tracking individual fish in open water seems feasible considering recent advances and applications made in the field of underwater acoustic telemetry (for examples, see Wroblewski et al. 1996, Starr et al. 2000, and Thorstad et al. 2000). However, it would also be necessary to change the thyroidal status for an extended period of time. The thyroidectomy procedure is practically impossible in most teleosts because the thyroid gland consists of many follicles irregularly dispersed in the connective tissue (Woodhead 1975). However, it may be possible to create sustained experimental hyperor hypothyroidism by osmotic pump delivery of thyroid or anti-thyroid agents. The advantage of using surgically implanted osmotic pumps is that they continuously deliver test solutions at controlled rates for several weeks, thereby eliminating the need for repeated dosing. To date, osmotic pumps have been implanted intraperitoneally in aquaculturally important species to study the effects of various agents on growth (Down et al. 1989), reproduction (Marte et al. 1987, Matsuyama et al. 1995), and disease resistance (Kelly et al. 1993).

The objective of this study was to determine whether osmotic pump delivery of thyroid enhancing and thyroid inhibiting agents could modify the thyroidal status of cod held in their natural environment. More specifically, I tested whether osmotic delivery of T_3 could change the concentration of thyroid hormones in the bloodstream of cod. In the same way, I investigated the effects of three anti-thyroid drugs: potassium iodide, methimazole (also known as tapazole), and thiourea. In another experiment, I examined the effects of gonadal hormones (estradiol-17β, testosterone) on thyroidal activity, since gonadal hormones reportedly altered thyroidal status in some freshwater and anadromous species of fish (Cyr and Eales 1996).

METHODS

Experiment design

This study was carried out on southern Gulf of St. Lawrence cod, a population that resides inside the Gulf during summer but migrates distances up to 650 km (Hanson 1996) to overwinter in the Cabot Strait (Jean 1964, Campana et al. 1999). In September

1997, before the onset of the overwintering migration, 50 adult (fork length > 40 cm) cod were captured inside the Bay of Chaleurs (47°53'N, 65°37'W) using longline gear. The fish were tagged, measured and transferred into two identical 6 m^3 cages resting on the sea bottom at a depth of 25m. The fish were fed to satiation once a week with chopped Atlantic mackerel (*Scomber scombrus*). In early October, individuals were randomly assigned to one of six experimental groups of equal ($p > 0.05$, Kruskal-Wallis test) fork length and body weight. Fish were anaesthetized using a solution of tricaine methane sulfonate (40 mg l^{-1} seawater - Syndel International Inc., Vancouver, BC). Blood samples (2 - 3 ml) were drawn from the caudal vessel to determine whether thyroid hormone levels differed among the investigated groups before the start of the experiment. Alzet's (Alza Inc., Palo Alto, CA) osmotic pumps (model 2ML1, 2 ml reservoir, 1.4 × 5.1 cm) were then surgically implanted into the peritoneal cavity. The pumps were previously filled with a placebo solution (0.7% NaCl), a T_3 solution (18 mg ml^{-1}), or an anti-thyroid solution containing potassium iodide (350 mg ml^{-1}), methimazole (80 mg ml^{-1}) or thiourea (80 mg ml^{-1}). To obtain the desired doses, two pumps were implanted in the individuals treated with potassium iodide, and three pumps were implanted in those receiving methimazole or thiourea. Some fish were untreated to assess the effects of surgery and presence of pumps on the thyroidal status. Blood samples were again collected 17 days following the start of the experiment. At this time, the fish were killed and the volume of the solution remaining in the pumps was measured in order to back calculate the dosages received during the experiment.

Another experiment was undertaken to determine the effects of gonadal hormones on thyroidal status. The experiment adhered to a 2 × 2 factorial design with the following groups: control (0.9%NaCl), estradiol-17β, testosterone, and estradiol-17β × testosterone. In September 1998, 36 cod were captured by longline in the Bay of Chaleurs and immediately transferred into one of three cages moored on the sea bottom (26 m). As in the case of the 1997 experiment, the cod were fed to satiation once a week with chopped mackerel. There was no reason to suspect a cage effect since cages were identical in size (3 m^3), moored at the same location, and contained a similar number of individuals. Nonetheless, I decided to distribute individuals so that the four treatment groups would be represented in each cage. The Kruskal-Wallis test indicated that the cod in the different treatment groups had similar fork length and body weight. The treatment solutions were delivered using the same type of osmotic pumps as in 1997. The concentrations of estradiol-17β and testosterone solutions inside the pumps were 14 $\mu g\ ml^{-1}$ and 71 $\mu g\ ml^{-1}$ respectively. Blood samples were collected for the measurement of hormones 20 days following the implantation of osmotic pumps.

Bottom water temperatures were monitored during the experiments by securing automated temperature recorders inside the cages. The recorders (Minilogs, Vemco Ltd., Canada) logged temperature every hour with an estimated accuracy of 0.2°C.

Laboratory measurements

I determined the gonadosomatic index (I_G) as 100 × the weight of the gonads divided by the weight of the carcass (total body weight minus combined weight of liver, gonads, and stomach) (Schwalme and Chouinard 1999). Energy reserves were measured using the hepatosomatic index (I_H), calculated as in Lambert and Dutil (1997a):

$$I_H = (LW \cdot W^{-1}) \cdot 100$$

where LW represents the liver weight in grams and W is the somatic weight in grams. Somatic weight was calculated as the total weight of the fish minus the combined weight of gonads and stomach content.

Hematocrit was determined by centrifuging capillary tubes containing cod blood. Serum was obtained by allowing refrigerated blood to coagulate overnight in non-heparinized tubes. Serum samples were frozen in liquid nitrogen and stored at -72°C prior to radioimmunoassay analyses. I measured total (protein-bound and -unbound) T_4 and total T_3 using a combined radioimmunoassay (RIA) technique (Omeljianuk et al. 1984) and the reagents employed by Cyr et al. (1998). Details of the procedures are given in Comeau et al. (2000). Gonadal hormones were measured using commercial Coat-A-Count® RIA kits (Diagnostic Products Corporation, Los Angeles, CA). Radioactivity was quantified using a Beckman Gamma 5500 Counting System. The dilution of cod serum samples yielded hormone levels close to the predicted values, and the dilution curves of samples were parallel to the dilution curves of standards. Intra- and interassay coefficients of variation were less than 10%.

Statistics

Data were $\log_{10}$ transformed to ensure normality and homoscedasticity. After transformation, the samples that met the assumptions of normality and homogeneity of variances were compared using the t-test. In cases where the transformation was not effective, nonparametric tests (Kruskal-Wallis or Mann-Whitney) were applied to the untransformed data.

RESULTS

The water temperature inside the cages averaged 7.6°C (SE = 0.1°C) in 1997 and 5.2°C (SE = 0.2°C) in 1998. Under these conditions, the osmotic pumps delivered on average 3% (SE = 0.5%) of their initial volume per day. At this rate, the expected delivery duration or life expectancy of osmotic pumps is over 30 days.

In 1997, mean levels of thyroid hormones were similar among treatment groups prior to the implantation of osmotic pumps ($p > 0.05$, Kruskal-Wallis test). After a 17-d treatment with T_3, however, levels of thyroid hormones (T_4 and T_3) increased significantly over control values (Table 5.1). Potassium iodide had no measurable effect on levels of thyroid hormones. In contrast, T_3 levels were significantly depressed to about 45% of control levels in both the methimazole and thiourea treatment groups. Hematocrit and I_H (data not shown) values were similar among the investigated groups ($p > 0.05$, Kruskal-Wallis test). Also, I found no evidence that the presence of osmotic pumps inside the body cavity had an impact on thyroidal status. The mean concentrations of thyroid hormones in the untreated and placebo fish were similar ($p > 0.05$, t-test).

Table 5.1. Drug doses and concentrations of thyroid hormones in cod serum 17 days after the implantation of osmotic pumps in 1997. Values represent means (SE).

		Dosage	Thyroid Hormones in Serum		Hematocrit
			T_4	T_3	
Treatment	n	$\mathrm{mg\ d^{-1}}$	$\mathrm{ng\ ml^{-1}}$	$\mathrm{ng\ ml^{-1}}$	%
Untreated	5		4.7 (0.5)	58.0 (5.3)	30 (5)
Control					
0.7% Saline	10		7.4 (3.8)	67.7 (16.4)	28 (4)
Thyroid hormone					
T_3	9	1.2 (0.1)	42.2[‡] (9.0)	5,911[‡] (1,503)	29 (2)
Thyroid inhibitors					
Potassium Iodide	9	50.6 (2.0)	3.2 (0.4)	48.1 (2.4)	31 (3)
Methimazole	10	16.4 (0.3)	2.6 (0.5)	37.3[†] (5.2)	27 (2)
Thiourea	7	16.2 (0.3)	3.1 (0.5)	37.6[†] (7.5)	31 (2)

† $p < 0.05$ Significantly lower than controls (t-test, one tail)
‡ $p < 0.01$ Significantly different than controls (Mann-Whitney, two tails)

Serum estradiol-17β levels were similar among the treatment groups at the end of the 1998 experiment (Table 5.2), suggesting that the daily dose (0.9 µg) of estradiol-17β was relatively weak. However, mean testosterone levels tended to be higher in groups treated with 4 µg of testosterone per day. Mean I_G values in the various groups were low ($I_G < 2.0$), indicating post-spawning conditions. I found no correlations between the I_G values and testosterone levels. Thus, the elevated testosterone levels were probably due to the introduction of exogenous testosterone into the body cavity.

Table 5.2. Drug doses and concentrations of gonadal and thyroid hormones in cod serum 20 days after the implantation of osmotic pumps in 1998. Sexes are shown separately. Values are means (SE).

	n		Dosage $\mu g\ d^{-1}$		Mean Concentrations of Hormones in Serum $ng\ ml^{-1}$			
Treatment	♂	♀	Estradiol-17β	Testosterone	Estradiol-17β	Testosterone	T_4	T_3
Control (0.9% Saline)	4				0.04(0.01)	1.5(0.5)	3.5(1.2)	11.0(1.8)
Estradiol-17β	6		0.86(0.02)		0.22(0.13)	1.5(0.4)	3.4(0.8)	12.8(3.7)
Testosterone	5			4.3(0.1)	0.27(0.21)	4.5(1.7)	1.5(0.3)	14.4(1.6)
Estradiol-17β × Testosterone	6		0.86(0.02)	4.4(0.1)	0.27(0.15)	$3.6^{\dagger}(0.3)$	1.6(0.4)	13.8(1.5)
Control (0.9% Saline)		5			0.05(0.01)	1.1(0.3)	3.7(1.1)	11.3(1.2)
Estradiol-17β		4	0.86(0.04)		0.02(0.01)	0.7(0.1)	1.6(0.9)	8.2(2.6)
Testosterone		4		4.4(0.2)	0.02(0.01)	2.7(0.9)	$1.3^{\dagger}(0.3)$	14.4(1.8)
Estradiol-17β × Testosterone		2	0.88(0.09)	4.4(0.5)	0.08(0.03)	0.6(0.1)	2.7(0.3)	10.7(0.5)

† $p < 0.05$ Significantly different from controls (t-test, two tails)

Serum T_4 levels tended to be depressed in cod that received testosterone (Table 5.2). The effect was significant in females that received testosterone alone. In this group, mean T_4 was reduced to 65% of control values. Only two female cod were included in the estradiol-17β × testosterone treatment group, making it difficult to conclude whether or not there was an interaction effect between the two gonadal hormones. For males, the reduction in mean T_4 levels following testosterone treatment was non-significant. However, it is noteworthy that the mean T_4 levels were reduced by a similar magnitude (56 - 59%) in the two groups of males treated with testosterone (i.e., testosterone and estradiol-17β × testosterone groups).

DISCUSSION

This study demonstrated the value of osmotic pumps as effective delivery vehicles for drugs in wild demersal fish. Treatment with T_3 (1.2 mg d^{-1}) for seventeen days resulted in a severe hyperthyroid condition, with serum T_3 levels averaging 5,911 ng ml^{-1}. This concentration is no doubt supraphysiologic, since natural T_3 levels in cod serum vary between 2 and 27 ng ml^{-1} (Comeau et al. 2000). Hence, there is little doubt that exogenous T_3 released inside the peritoneal cavity by osmotic pumps can effectively enter the blood circulatory system and change the thyroidal status in wild cod. In future experiments, simply adjusting the concentration of T_3 in the delivery solution could bring T_3 within physiological boundaries. The reason for the parallel increase in T_4 is not known. Like cod, rainbow trout *Salmo gairdneri* treated with a supraphysiological T_3

dose exhibited high T_4 (Cyr and Eales 1988), suggesting that the high T_4 levels might reflect an abnormal adjustment in the peripheral thyroid system.

Both methimazole and thiourea rendered cod hypothyroid by depressing serum T_3 to about 45% of control values. In the fish literature, no method for completely blocking thyroid function has been described to date. For instance, when rainbow trout were exposed to anti-thyroid drugs by injection or immersion, plasma T_3 fell to 40% of control levels (Cyr and Eales 1986, 1988). Moreover, the mode of action of thyroid inhibitors in fishes is not fully understood. Since methimazole and thiourea caused no significant depression of serum T_4, it may be inferred that the drugs had no effect on the release of T_4 by the thyroid gland. The inhibitors could have acted mainly on the peripheral thyroidal system, possibly inhibiting the extrathyroidal deiodination of T_4 to T_3.

The inhibitors exerted no conspicuous extrathyroidal effects, assessed by hematocrit and I_H values (Eales 1981, Cyr and Eales 1986). Nevertheless, Eales (1981) and Cyr and Eales (1996) emphasized the importance of taking into consideration extrathyroidal effects when designing experiments involving thyroid inhibitors. Therefore, with respect to the open water tracking experiment described earlier, investigators could compare the migration rates of thyroid-inhibited cod with the migration rates of thyroid-inhibited cod also treated with thyroid hormones (replacement therapy).

The low concentrations of estradiol-17β measured in cod serum at the end of the treatment period suggest that the estradiol-17β treatment (0.9 $\mu g\ d^{-1}$) may have been too weak to impact endocrine status. For that reason, it is difficult to reach any conclusions about the effect of estradiol-17β, or about the possibility of an interaction between estradiol-17β and testosterone. By comparison, it appears that the testosterone treatment (4 $\mu g\ d^{-1}$) effectively increased testosterone levels. It is unlikely that the differences in testosterone levels among the groups were due to variations in secretion rates by the gonads because the majority of individuals were in post-spawning condition ($I_G < 2.0$, for annual I_G cycle see Schwalme and Chouinard 1999).

I found that T_4 levels were significantly depressed in females treated with testosterone during 20 days. A similar but non-significant reduction in thyroidal status occurred in males treated with testosterone. An inhibitory effect of testosterone is contrary to reports on freshwater and anadromous species, for which the administration of androgens tended to elevate thyroidal status (Cyr and Eales 1996). However, the inhibitory effect of testosterone is consistent with field observations on southern Gulf cod: at peak spawning time (June), a rise in testosterone levels coincided with a decline in T_4 levels (for gonadal hormones, see Comeau et al. in press; for thyroid hormones, see Comeau et al. 2000).

I conclude that the administration of T_3 by osmotic pumps is an effective means for creating experimental hyperthyroidism in wild cod. Similarly, the administration of methimazole, thiourea, or testosterone with osmotic pumps was effective for rendering cod hypothyroid. Based on the type of osmotic pumps tested in the current study, the duration of the thyroidal effects could be extended to periods over 30 days. These results should be useful for future experiments concerned with the release of hyper- or hypothyroid cod in open water.

CHAPTER SIX

Timing of Atlantic cod (*Gadus morhua* L.) seasonal migrations in the southern Gulf of St. Lawrence: interannual variability and proximate control

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ABSTRACT

The aim was to identify likely proximate cues to the seasonal migrations of cod *Gadus morhua* in the southern Gulf of St. Lawrence. I computed a migration timing index across several years using commercial fishery (1970-1992) and sentinel survey (1995-1999) catch rate data, and closely monitored the seasonal changes in temperature. dissolved oxygen, food intake, and energy stores between 1995 and 1998. The inter-year variability in the timing of the seasonal migrations was relatively low (standard deviation = 8 d for spring migration, 10 d for autumn migration), suggesting that photoperiod was involved in the control mechanism of migration. However, other factors were also implicated in the control mechanism, given that cod initiated the autumn migration progressively earlier in the late 1980s and 1990s. At the onset of the autumn migration. dissolved oxygen concentrations were above levels known to induce avoidance behaviour in this species, and food resources were considered to be relatively abundant. Similarly. cod did not initiate the autumn migration at a specific temperature or following a particular temperature regime experienced during the previous summer. However, cross correlation function analysis indicated that a widespread cooling of near bottom waters

preceded the mid-1980s shift in migration timing by 1 to 2 years, suggesting that the cooling event was linked in some way to the change in migration dates. Other correlations showed that the shift in timing coincided with a reduction in growth rates and also with a rise in the abundance of fish prey inside the southern Gulf. I propose that a reduction in metabolic requirements coupled to an increase in the consumption of fish prey led to a rapid build-up of energy reserves on summer grounds, and thus to an earlier readiness to engage in the autumn migration.

INTRODUCTION

Many of the world's most commercially important cod (*Gadus morhua*) populations perform extended seasonal migrations (up to 1000 km, Woodhead 1975). They generally move to inshore areas in spring, and return to offshore waters in autumn (McKenzie 1956, Jean 1964, Harden Jones 1968, Woodhead 1975, Templeman 1979, Lear and Green 1984). Presumably, the spring migration provides individuals with good feeding opportunities in shallow inshore areas (e.g., see Ponomarenko 1996, Lambert and Dutil 1997b, Schwalme and Chouinard 1999, O'Driscoll et al. 2000). In autumn, cod may return offshore to avoid what will ultimately be near freezing waters in their summer habitat. While cod can produce plasma antifreeze glycopeptides (Hew et al. 1981, Fletcher et al. 1987), adult cod do not have the capacity to survive water temperatures below about -1.2°C (Kao and Fletcher 1988).

To ensure that the evolutionary benefits of migration outweigh the risks, a safe and reliable control mechanism must be in place so that migratory movements are initiated at the appropriate times within the physiological and environmental cycles. However, our current understanding of the control mechanism of cod migration is limited. For instance, the question as to whether the migrations are cued to the seasonal changes in water temperature remains open. Temperature has been correlated with cod distribution in several studies (Jean 1964, Scott 1982, Rose and Leggett 1988, Smith et al. 1991, Swain and Kramer 1995, Castonguay et al. 1999), and temperature changes alone were shown to modify swimming behaviour in captive cod (Claireaux et al. 1995a). Yet, empirical analyses relating *in situ* temperature changes to actual onset of migration are lacking, although such reports already exist for anadromous species (Jonsson and Ruud-Hansen 1985, Bohlin et al. 1993). Other plausible but yet untested environmental factors that may cue cod migrations include photoperiod (Woodhead and Woodhead 1965), dissolved oxygen concentrations (D'Amours 1993), and food supply (Templeman 1965). With respect to food supply, an alternate hypothesis is that an annual cycle in appetite (see Fletcher 1984, Wootton 1990) regulates migrations to and from feeding grounds. Similarly, it may be that internal changes in temperature preference (Clark and Green 1991, Despatie et al. in press), as opposed to environmental changes in temperature, trigger cod migrations.

Identifying the proximal cues to migration and thus being able to predict the onset of seasonal migrations has practical applications for fisheries management, particularly in cases where the timing of migrations are used to set the duration of mixed-stock fisheries.

The southern Gulf of St. Lawrence cod stock (Figure 6.1) provides a striking example in which seasonal migrations result in a mixed-stock fishery. In summer, southern Gulf cod are found inside the Gulf of St. Lawrence [Northwest Atlantic Fisheries Organisation (NAFO) division 4T] (Jean 1964, Paloheimo and Kohler 1968). In autumn, however, they migrate distances up to 650 km (Hanson 1996) to overwinter along the southern edge of the Laurentian Channel in the Cabot Strait (NAFO 4Vn) and the eastern Scotian Shelf (NAFO 4Vsb) where they are mixed with stocks from these areas (Lambert 1993, Campana et al. 1999). In spring, they migrate back into NAFO 4T, thus completing their annual migration cycle.

My objective was to identify the likely variable(s) cueing the seasonal migrations of southern Gulf cod. As a first step in this process, I estimated the timing of seasonal migrations for the years between 1970 and 1999. I then assessed whether the seasonal migrations are relatively constant from year to year, a result that would support a photoperiod trigger mechanism. I also compared migration timing with changes in environmental temperature and dissolved oxygen, and with the naturally occurring cycles in food intake, energy storage, and prey abundance on summer grounds. Finally, I propose a model linking the onset of seasonal migrations to both the environment and physiological rhythms.

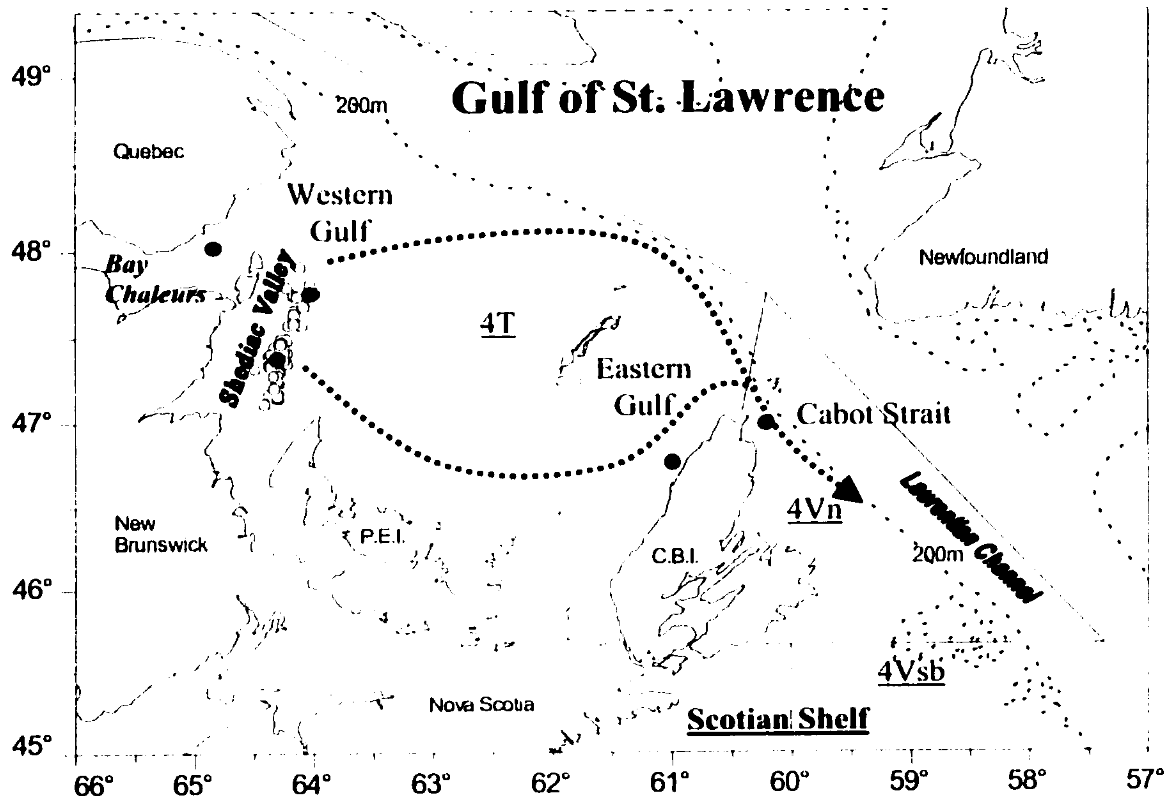

Figure 6.1. Likely migration routes (Sinclair and Currie 1994, G. A. Chouinard, unpublished data) between the southern Gulf of St. Lawrence (summer) and the Cabot Strait (winter) grounds. The ● symbols show the locations of temperature recorders deployed on sea bottom. The ○ symbols show sites where near bottom water samples were collected for measurement of dissolved oxygen. NAFO divisions 4T, 4Vn, and 4Vsb are shown.

MATERIAL AND METHODS

Migration timing

I used three mobile gear (seine and otter trawl) datasets to infer migration timing: personal logbooks, official logbooks, and sentinel surveys. The 'personal logbooks' are those that skippers traditionally maintain to document hook-up areas and catches; they are not the type submitted to regulatory agencies such as the Canadian Department of Fisheries and Oceans (DFO), and therefore not subject to intentional misreporting. I was successful in obtaining the personal logbooks from 11 different vessels. These logbooks represented a total effort of 30,681 sets carried out in the eastern Gulf of St. Lawrence between 1970 and 1992, after which there was a moratorium on the directed cod fishery. For any given season and year, only some of the 11 vessels in the dataset were useful to infer migration timing: the number of vessels that made frequent trips into the eastern Gulf of St. Lawrence ranged from a single vessel (1970-1972; 1979-1980) to ten vessels (1990), with the 1970-1992 mean being equal to four (SE = 0.5) vessels. In addition to their personal notes, skippers maintain a separate set of logbooks, commonly termed 'official logbooks' that are regularly submitted to DFO. The available dataset covers a relatively short period (1986-1992) but includes many vessels (mean = 20) for any given season and year in both the eastern and western Gulf. Lastly, sentinel surveys helped determine the timing of the autumn migration from 1995 to 1999. Sentinel surveys consist of periodic sampling excursions on traditional fishing grounds following a standardized protocol. I used the data from two sentinel survey vessels in the western Gulf, and two other vessels in the eastern Gulf.

In keeping with the understanding that migrating cod form large and dense aggregations (Rose 1993), which make them more vulnerable to mobile fishing gear, I used the day of peak catch rates as an index of migration timing. As a first step in the calculation of the timing index, trip or daily means of catch rates were produced for each vessel. The means reflected a substantial effort: about 10 sets per trip for the sentinel series, 13 h per day for the official logbooks, and five sets per day for the personal logbook series. Then, for each vessel, the time series of catch rates was visually inspected for a single peak in either spring or autumn. The peak in catch rate was generally unambiguous. For instance, when compared with the previous three days on record, the peak represented an increase in catch rate of 190% (SE = 25%) in spring and 246% (SE = 27%) in autumn (based on personal logbooks). Finally, for any given season and year, a mean date corresponding to peak catch rate was computed using all available vessels. With respect to the spring timing index, I excluded the years characterized by late ice retreat (e.g. 1991 and 1992), since a delayed start of the fishery would have skewed the index.

Environment

Bottom water temperatures were monitored between 1995 and 1998 by deploying temperature recorders at five locations within the study area (Figure 6.1, filled circles). The recorders (Minilogs, Vemco Ltd., Canada) logged bottom temperature every 3 h with an estimated accuracy of 0.2°C. Weekly means and standard deviations (SD) were calculated for each site separately. In the western Gulf, near bottom water samples were regularly collected at two to five sites arbitrarily selected for dissolved oxygen measurements (Figure 6.1, open circles). Oxygen concentrations were measured in duplicate using the Winkler titration procedure (Aminôt and Chaussepied 1992). Oxygen concentrations are reported as trip means.

Fish collection and nutrition indices

A total of 1,613 adult (fork length = 52.5 ± 0.3 cm) cod were collected as part of research and sentinel survey expeditions in 1995, 1996 and 1997. The number of fish collected each month ranged from 21 to 209 and both sexes were generally well represented. The sampling interval ranged from one to two weeks during the summer and autumn and from one to two months during the winter and spring. The exact sampling area varied according to the seasonal migrations of the cod. During the summer and autumn, samples were collected inside the Gulf of St. Lawrence, particularly in the western region where a large fraction of the population resides at this time of year (Chouinard et al. 2000). During the winter and spring, samples were collected in the Cabot Strait along the southern edge of the Laurentian Channel.

Relative (to body mass) food intake was reported as the stomach fullness index, representing the weight of the stomach content as a percentage of the carcass weight (Schwalme and Chouinard 1999). The carcass weight was calculated as the total weight of the fish less the combined weight of the liver, gonads, and the stomach with content. To provide an estimate of absolute food intake, I computed a standardized (50 cm fork length) stomach content weight index analogous to that of the Fraser-Lee procedure described in Carlander (1981) and Campana (1990). For each trip, I produced a regression equation relating the stomach content weight and fork length. The regression intercept (*b*) was then incorporated into the following equation to determine the standardized stomach content weight:

$$Stom_{50cm} = b + (Stom_c^{-1} - b) \cdot L_c \cdot 50$$

where $Stom_{50cm}$ is the standardized stomach content weight, L_c is the fish length at capture, and $Stom_c$ is the weight of the stomach content at capture. Energy reserves were measured using two indices, the Fulton's condition factor (*K*) and the hepatosomatic index (I_H). Both indices were calculated as in Lambert and Dutil (1997a):

$$K = 100 \cdot W \cdot L^{-3}$$

$$I_H = (LW \cdot W^{-1}) \cdot 100$$

where *W* represents the somatic weight in grams, *L* is the fish length in centimetres, and *LW* is the liver weight in grams. Somatic weight was calculated as the total weight of the fish minus the combined weight of gonads and stomach content.

Statistics

Sample means were compared using nonparametric statistics: the Mann-Whitney test for two samples, and the Kruskal-Wallis test for several samples. Trend lines were fitted to time series using either an iterative locally weighted least-squares (LOWESS) method or a centred moving average approach. In the latter case, missing data points in series were replaced with computed values (linear interpolation) prior to the calculation of the moving averages. Cross-correlation function (CCF) analysis was used to examine whether changes in migration timing lagged environmental changes. Standard correlation analysis was used to quantify the extent to which migration timing co-varied with other factors. I tested for autocorrelation by correlating series with lagged values of itself. I accounted for autocorrelation, when present, by computing an effective sample size (n_{eff}) in correlation analyses. This corrective procedure reduces the chances of incorrectly concluding that a correlation is significant (Type I error). The equations used to compute n_{eff} are those found in Dutil et al. (1999, equation 1 and 2) and incorporate the weighting factor proposed by Pyper and Peterman (1998). Note also that the corrective procedure requires the use of continuous time series. Because of this restriction, I had to limit my analyses to years between 1979 and 1992 (instead of 1970-1992) when accounting for autocorrelation.

RESULTS

Migration timing

The patterns in peak catch rates were consistent with the formation of large, dense aggregations prior to migration between summer and winter areas. In spring, the peaks were recorded first in the eastern Gulf, and subsequently in the western Gulf (Figure 6.2a, 6.2b); in autumn, a reverse spatial pattern was obvious when cod migrated out of the Gulf (Figure 6.2c, 6.2d). The time difference between the appearance of the western and eastern peaks provided an estimate of the duration of the migrations inside the Gulf. From 1986 to 1990 (official logbooks), the time difference between the two regional

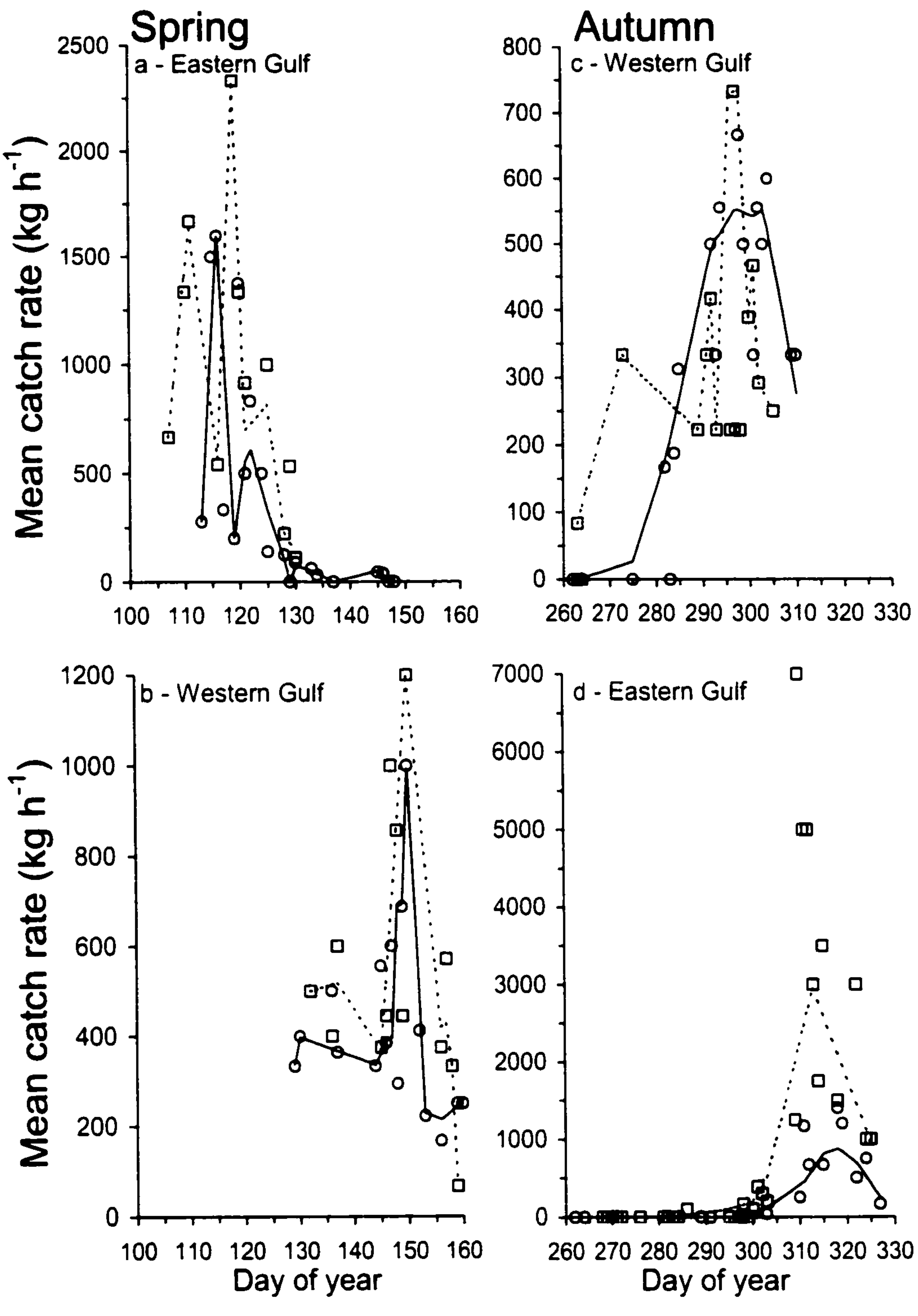

Figure 6.2. Examples of the changes in catch rates in spring (1990) and autumn (1989). Data points are from the official logbook series and represent daily means of individual mobile gear vessels (two vessels, ○ and □, shown per graph). Trend lines show locally weighted least-squares regression.

peaks was on average 25 d (SE = 7 d) in spring and 25 d (SE = 4 d) in autumn. From 1995 to 1999, sentinel surveys in autumn also showed a duration of migration of 25 d (SE = 4d).

In the eastern Gulf, the peak catch rates in spring were logged on May 3 with a standard deviation of only 8 days between 1970 and 1992 (Figure 6.3a, filled cycles, personal logbooks). The autumn timing index was slightly more variable (SD = 10 d), and more importantly, started a declining trend in 1986 (Figure 6.3b, filled circles, personal logbooks). Peak catch rates were logged on November 30 in 1970-1985 and on November 14 in 1986-1992. The 16 d difference between the two periods was highly significant (Mann-Whitney test, $p < 0.001$). In more recent years (1995-1999), peak catch rates in sentinel surveys were recorded on November 1. The November 14 and November 1 means were also significantly different (Mann-Whitney test, $p < 0.01$).

I examined the possibility that the declining trend since 1986 was due to changes in the fishing fleet composition, since the number of vessels included in the personal logbook dataset tended to increase with time. A small subset (n = 2) of vessels that fished most years during the entire period was thus inspected: the outcome was similar, with a definite tendency for earlier timing in 1986-1992. Peak catch rates occurred on average 13 d earlier than in 1970-1985 (Mann-Whitney test, $p < 0.02$). Moreover, there were no strong indications that the early peaks were caused by the shortening of the autumn fishery. The peaks during the 1986-1992 period were logged on average 7 d (SE = 1 d) before the last fishing trip in autumn and, perhaps more importantly, there was a tendency

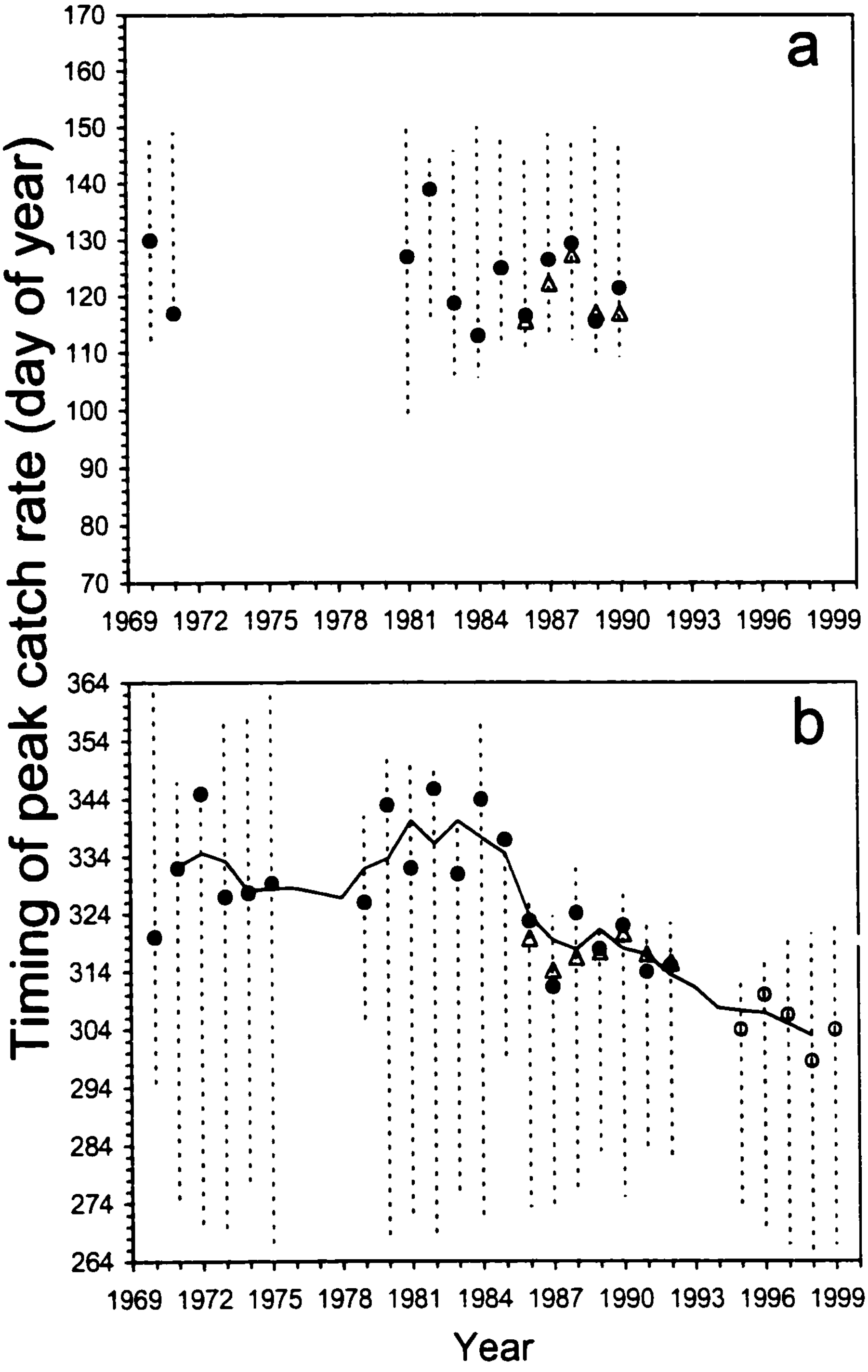

Figure 6.3. Timing of the spring (a) and autumn (b) peaks in catch rate in the eastern Gulf of St. Lawrence. ● = personal logbook series, Δ = official logbook series, ○ = sentinel series. Vertical dotted lines show duration of autumn fishery: lower limit marks the average day when boats began fishing within season, and upper limit shows the average day when the fishery ended within season. Trend lines show three-year running mean.

towards low or null catch rates soon after the peak catch rate event. The tendency for catch rates to be at a minimum in early autumn, and thus for early departure of cod, has been obvious since 1986 in both commercial and sentinel surveys. In 1990, for instance, the catch rates from two commercial vessels quickly fell in early autumn (Figure 6.4); by comparison, in 1982, the catch rates from the two same vessels remained elevated in early autumn. Finally, it does not appear that the early timing results since 1986 were due to a shift in the fishing area. Figure 6.5 shows that vessels recorded their peak catch rates consistently in the same region during the study period.

Migration in relation to the environment, food intake, and energy storage

In spring (1997-1998), the bottom water temperatures inside the Gulf increased above the lower limit for cod distribution (~ -0.5°C, Rose and Leggett 1988, Swain and Kramer 1995) (Figure 6.6). The thermal barrier first disappeared in the eastern Gulf, which is consistent with the spring migration route. The breakdown of the thermal barrier also coincided with the historical (1986-1990) peaks in catch rates (see *EG* and *WG* in Figure 6.6). In autumn (1995-1998), cod departed the western Gulf when temperatures were increasing (Figure 6.7a, b, c). For each recording station, the temperature at the onset of the autumn migration was significantly different between years ($p < 0.0001$, Kruskal-Wallis test applied to weekly samples). The temperature in any of the three weeks preceding the onset of migration was also different between years ($p < 0.0001$, Kruskal-Wallis test applied to weekly samples). Moreover, migration timing showed no consistent pattern in relation to within-week temperature variability (Figure 6.7d, e, f), and did not coincide with a specific number of degree-days (Table 6.1).

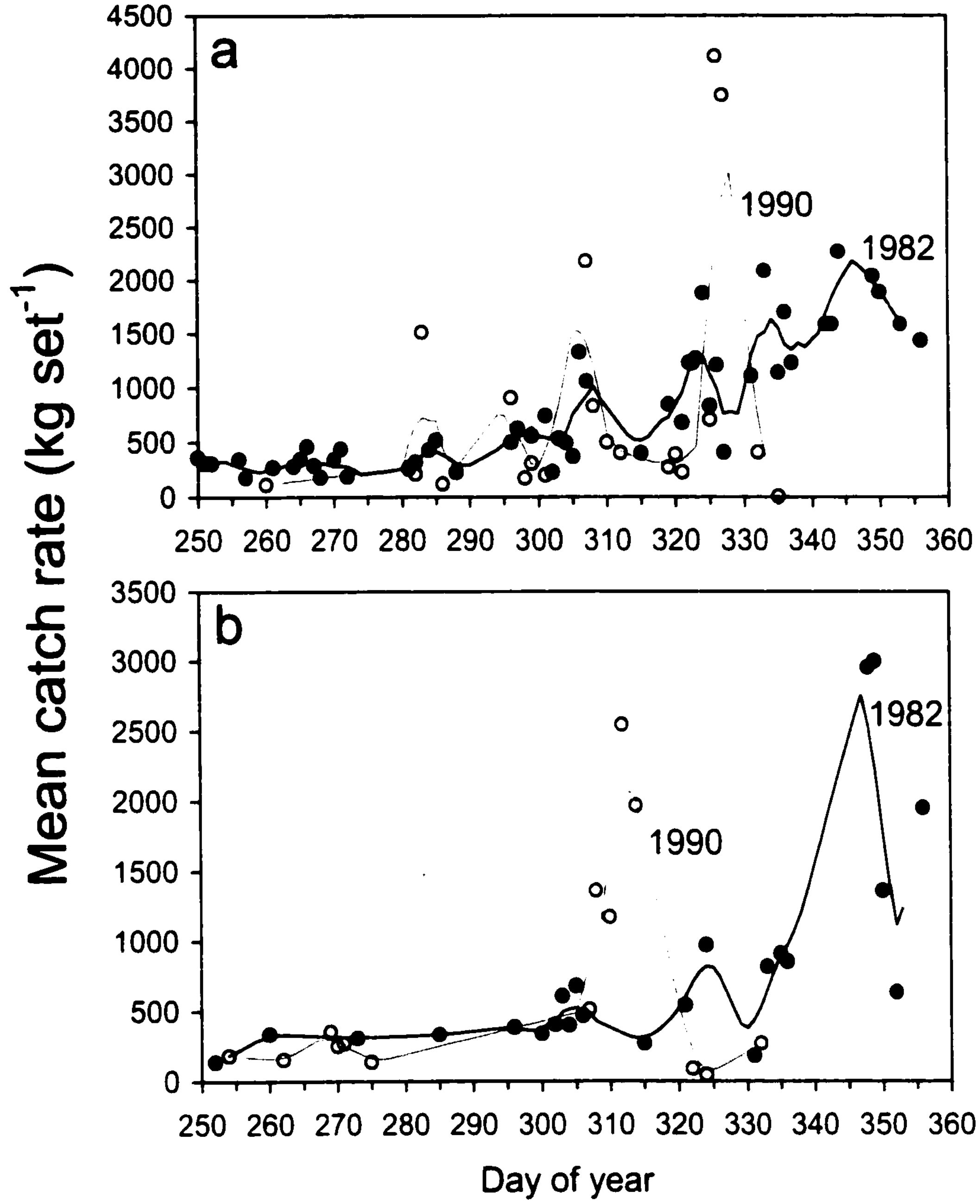

Figure 6.4. 1982 (○) and 1990 (●) autumnal catch rates (daily means) in the eastern Gulf. Graph (a) represents one fishing vessel and graph (b) another vessel, both from the personal logbook series. Trend lines show five-day running mean.

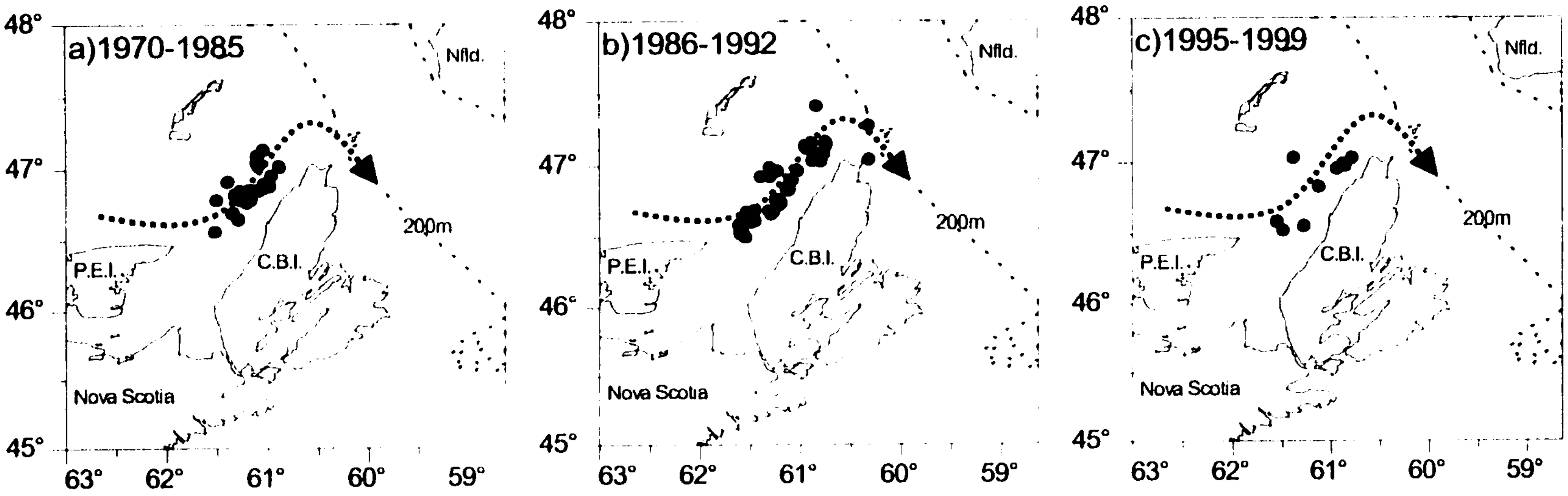

Figure 6.5. Locations (●) of autumnal peaks in catch rates for individual vessels. Personal logbook data shown in panels a and b; sentinel data shown in panel c. Arrow line shows likely migration route in autumn.

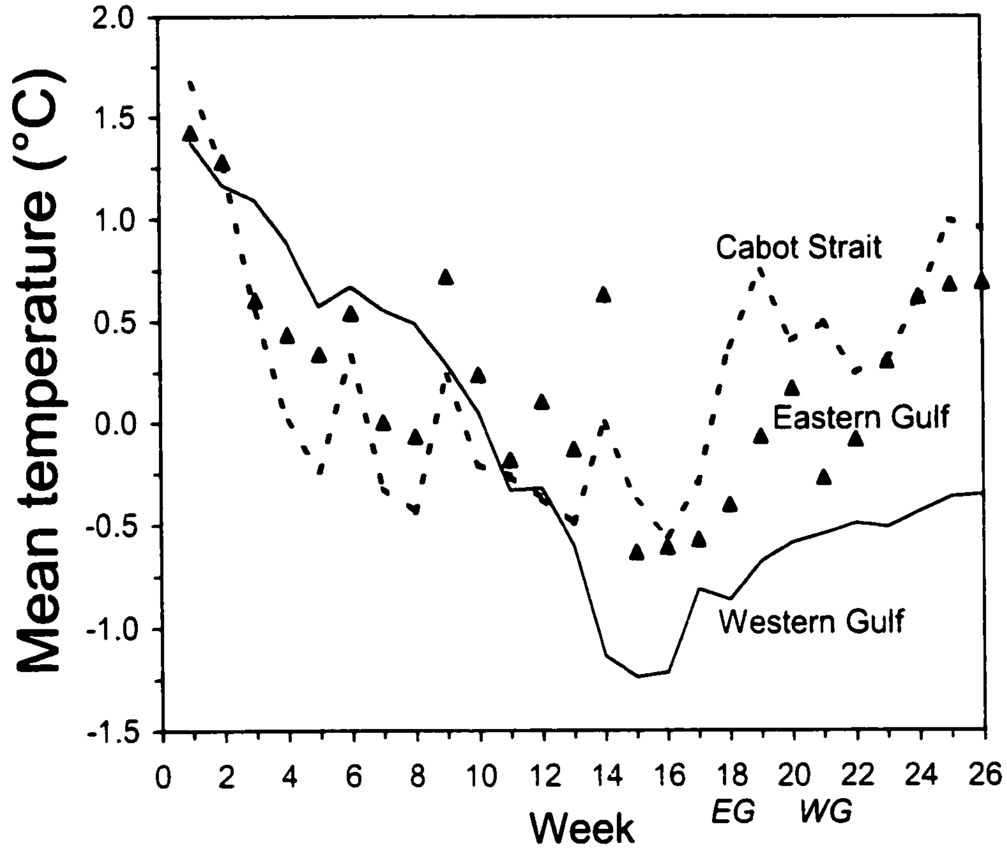

Figure 6.6. Weekly mean (1997-1998) bottom water temperatures in the western Gulf (—), eastern Gulf (▲) and Cabot Strait (----) during the first half of the calendar year. *EG* and *WG* indicate week when catch rates peaked in the eastern and western Gulf respectively (official logbooks, 1986-1990). Depth of temperature recording stations ranged from 84 to 88m.

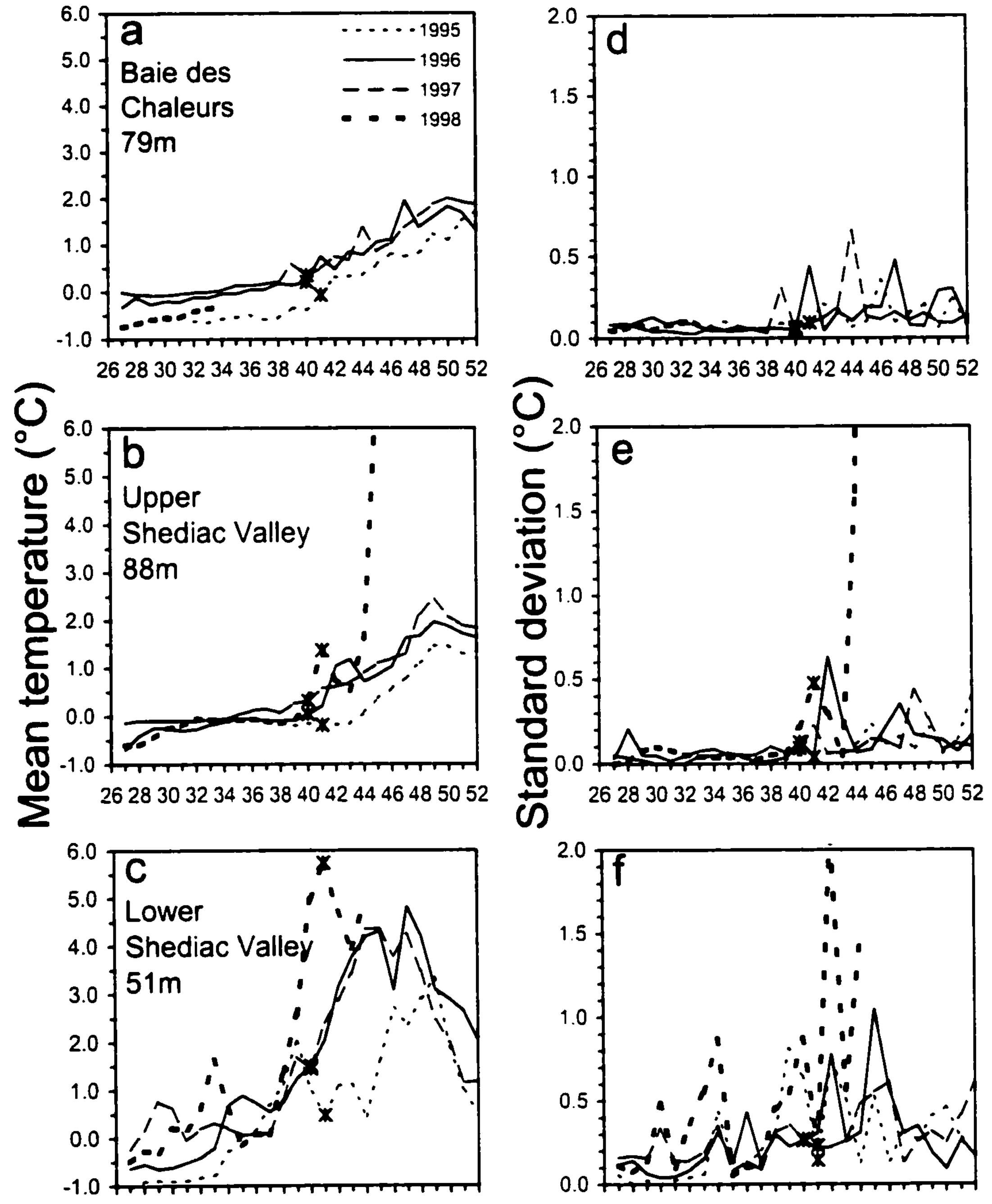

Figure 6.7. Weekly mean bottom water temperatures [(a), (b), (c)] and corresponding standard deviations [(d), (e), (f)] during the second half of the calendar year. Star symbols indicate week when catch rates peaked in sentinel surveys.

Table 6.1. Degree-days between July 1 and onset of migration in the western Gulf. Calculated using data from moored temperature recorders in upper and lower Shediac Valley.

	Upper Shediac Valley				***Lower Shediac Valley***			
	Number of days with temperature (°C) above							
Year	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1995	no data				43	27	14	6
1996	3	0	0	0	49	39	17	3
1997	31	0	0	0	72	33	11	6
1998	15	7	3	2	67	42	36	27
Range	28	-	-	-	29	15	25	24

With respect to dissolved oxygen, a trend towards low concentration values was evident on summer grounds. However, the lowest concentrations occurred about 30 d prior to the onset of the autumn migration (Figure 6.8). Oxygen concentrations were increasing and ranged between 8 and 9 mg ml^{-1} when cod departed the western Gulf.

Between 1995 and 1997, the stomach fullness index indicated that cod were feeding intensively as they entered the Gulf in spring, but that they fed little while leaving the Gulf in autumn (Figure 6.9). Condition indices followed an opposite trend. The condition factor and I_H values were low as cod moved into the Gulf in spring, but progressively increased on summer grounds and eventually peaked in autumn. The I_H, in particular, consistently peaked at (or just prior to) the onset of the autumn migration, and the magnitude of the peaks was similar among the investigated years (Kruskal-Wallis test, $p > 0.05$).

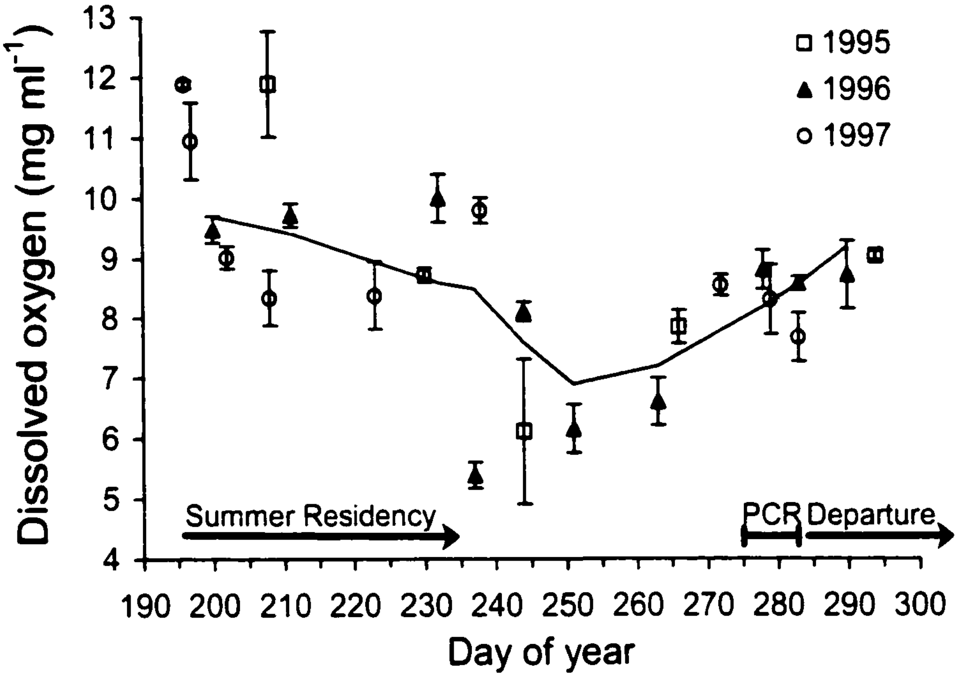

Figure 6.8. Bottom water oxygen concentrations in the western Gulf. PCR = Peak Catch Rates. Mean of 2 to 5 sites and corresponding SE bars are shown. Sampling depths varied from about 40 to 88m. Locally weighted least-squares line was fitted to 1996 data.

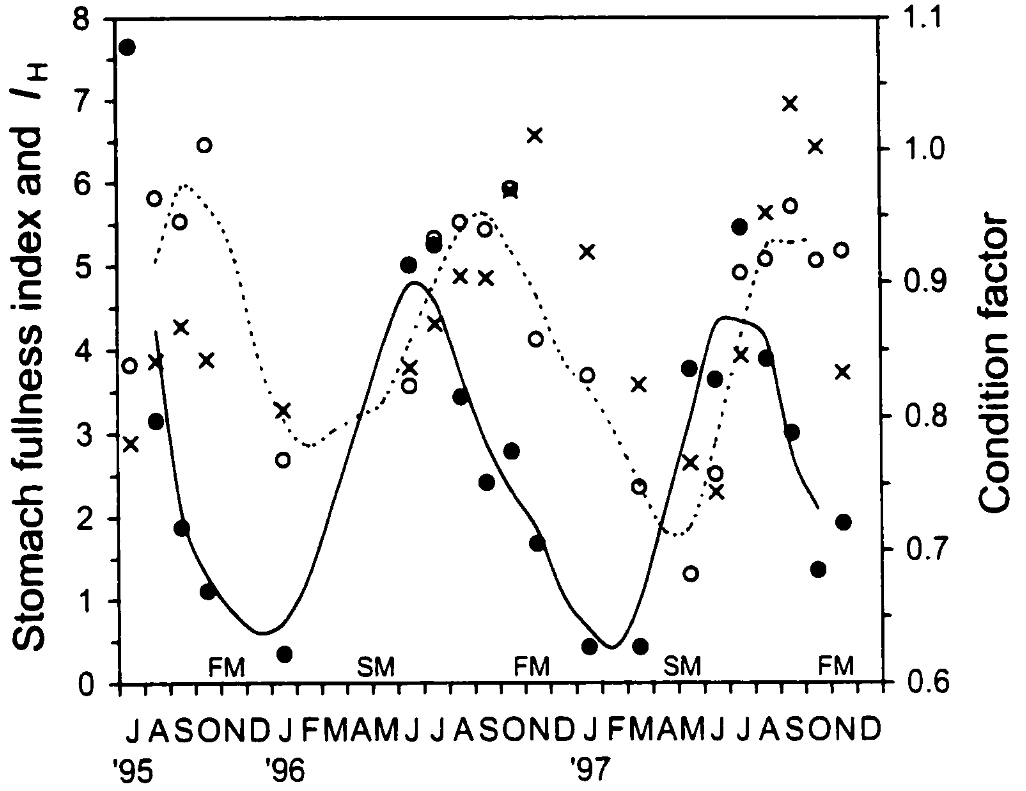

Figure 6.9. Seasonal changes in the stomach fullness index (•, —), I_H (○, ---), and condition factor (×, no trend line). *FM* = Fall Migration; *SM* = Spring Migration. Trend lines represent three-point centred moving averages.

A cruise-by-cruise analysis for the western Gulf provided further insight into the relationship between food intake, energy reserves, and the autumn migration. From 1995 to 1997, the stomach content weight represented between 6 and 7 % of the carcass weight at the beginning of summer, but only between 1 and 2 % at the onset of the autumn migration (see 'PCR' Figure 6.10a). Absolute stomach content weight showed a similar trend: in 1995, for instance, the stomach content weight of a 50 cm cod declined from 81 g (SE = 9 g) in early summer to only 8 g (SE = 4 g) at the onset of migration. Interestingly, a small fraction of the population was still present in the western Gulf in late October. In this group, food intake indices were significantly higher compared to cod sampled in early October (Table 6.2), while condition indices, particularly the I_H, were relatively low. Therefore, the late departing cod were in poor condition but feeding intensely.

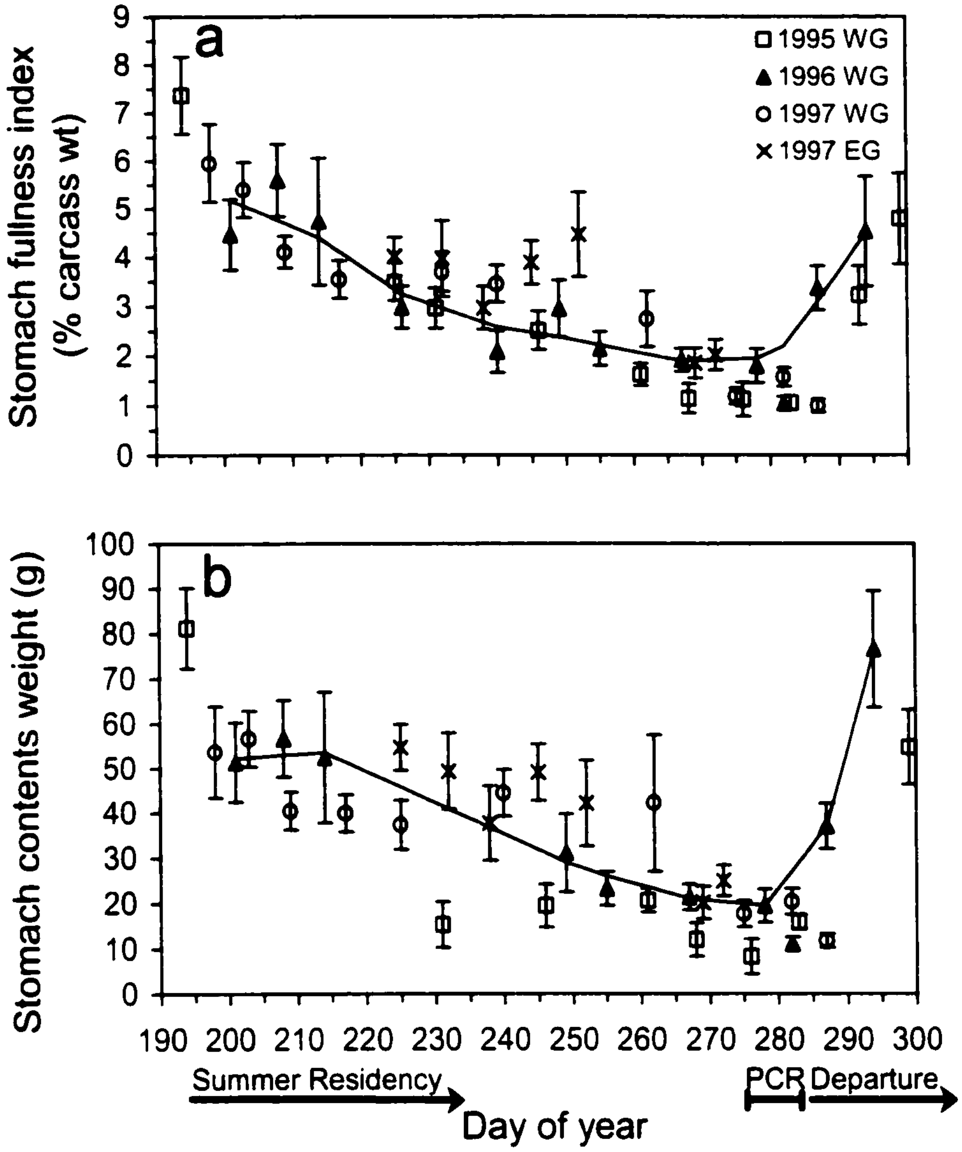

Figure 6.10. Stomach fullness index (a) and standardized stomach content weight for 50 cm cod (b) in western Gulf (WG) and eastern Gulf (EG). PCR = Peak Catch Rates. Locally weighted least-squares line was fitted to 1996 data.

Table 6.2. Food intake and energy reserves in western Gulf cod collected in early and late October. Values are expedition means.

	1995		1996	
	Oct 10	Oct 26	Oct 5	Oct 20
Food intake				
Stomach content weight (g)	15.8	54.6^{***}	19.5	76.1^{***}
Stomach fullness index	1.1	4.8^{***}	1.8	4.5^{***}
Energy reserves				
Carcass weight (g)	1015	978^{**}	1009	1010^{ns}
Condition factor	0.92	0.87^{*}	0.90	0.84^{ns}
I_H	6.3	4.6^{**}	6.6	3.1^{***}

Note: The number of fish in samples ranged from 14 to 30. The carcass and stomach content weights were standardized for a 50 cm cod. Stars indicate significant difference between the two October samples (Mann-Whitney: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = non-significant). Data from 1997 have been omitted due to poor coverage in late October.

In the longer term (1970-1992), the autumn migration tended to occur earlier as temperatures declined in the cold intermediate layer (CIL) (Figure 6.11a). However, the correlation between the two variables was non-significant, even after the removal of the 1984 outlier. CCF analysis suggested that changes in migration schedules lagged behind changes in temperature. Significant correlations were found only when the timing index lagged the CIL index (Figure 6.11a) by one year ($r = 0.51$, $n = 19$, $p < 0.05$) and the bottom water temperatures (Figure 6.11b) by two years ($r = 0.62$, $n = 17$, $p < 0.01$). There was no significant correlation between timing and the areal extent of cold bottom waters (Figure 6.11c).

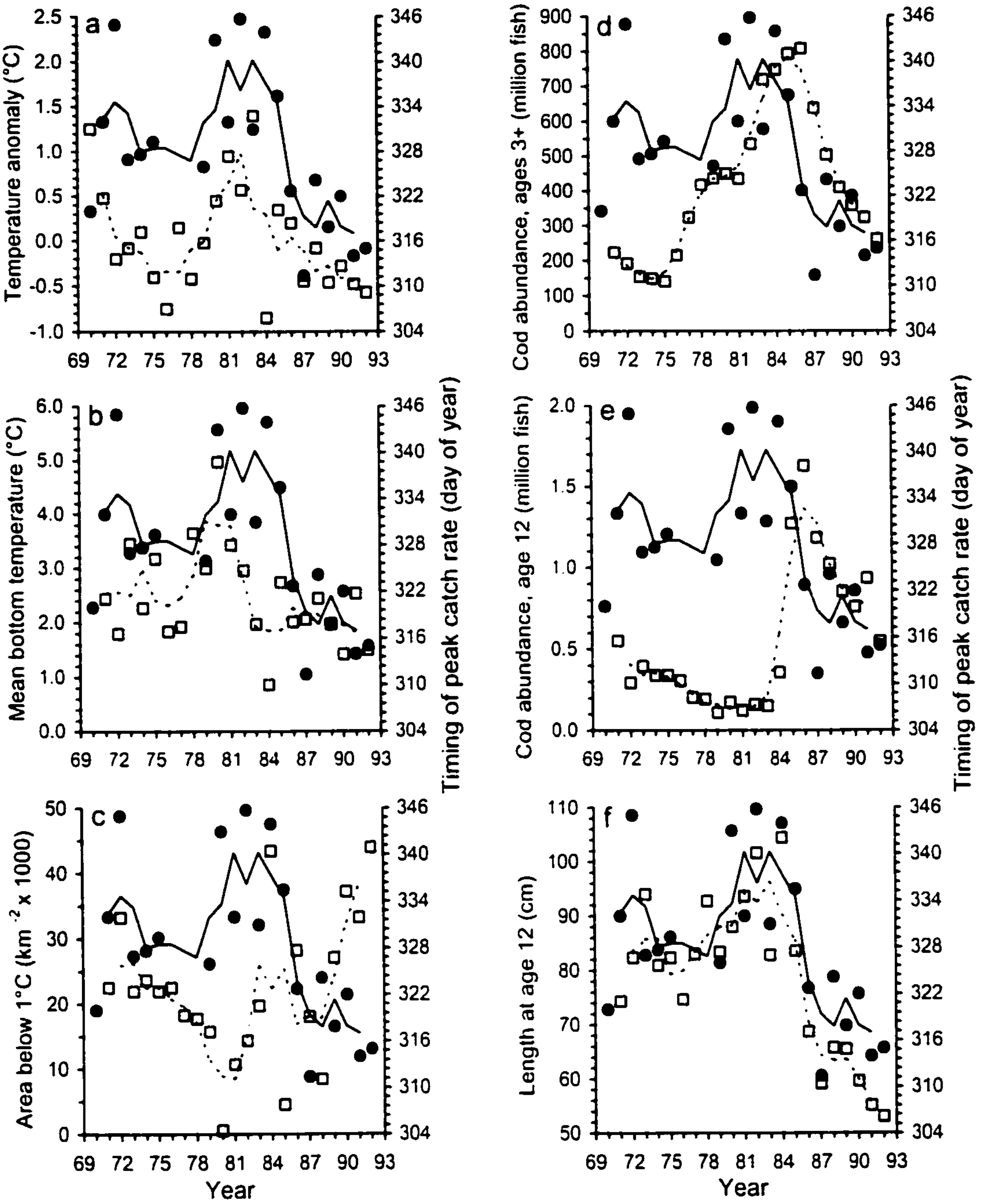

Figure 6.11. Timing of autumnal peaks in catch rates in the eastern Gulf (●, personal logbook series) in comparison to long term temperature anomalies (□, graph a), mean bottom temperatures (□, graph b), areal extent of cold bottom waters (□, graph c), cod abundance (□, graph d, e), and length at age (□, graph f). The temperature anomalies represent deviations from the 1948 - 1994 mean temperature minimum in the CIL (taken from Gilbert and Pettigrew 1997, southern Gulf, extrapolated to July 15). Mean bottom temperatures and areal extent of cold waters data were taken from Swain (1993) and reflect the thermal conditions during the month of September. Population abundance and length at age data were taken from Chouinard et al. (1999a). Trend lines show three-year running mean.

I next examined whether the changes in migration timing were correlated with changes in cod population attributes. For these analyses, the data were first $\log_{10}$ transformed to create normal distributions and improve linearity in correlations. This transformation did not remove autocorrelation, and consequently an effective sample size was calculated for statistical inference. No significant correlations were found between timing and cod population abundance (Figure 6.11d). However, negative correlations were found between timing and the abundance of older cod, namely those of age 13 ($r = -0.76$, $n = 14$, $n_{eff} = 6$, $p < 0.05$, Figure 6.11e) and age 14 ($r = -0.66$, $n = 14$, $n_{eff} = 7$, $p < 0.05$). In the same way, timing was positively correlated with the body size (fork length) of older cod only, specifically those of age 11 ($r = 0.71$, $n = 14$, $n_{eff} = 6$, $p < 0.05$), age 12 ($r = 0.93$, $n = 14$, $n_{eff} = 6$, $p < 0.01$, Figure 6.11f), and age 13 ($r = 0.72$, $n = 14$, $n_{eff} = 7$, $p < 0.05$). It is also noteworthy that migration timing was negatively correlated with the abundance of herring (*Clupea harengus*) and capelin (*Mallotus villosus*) inside the southern Gulf of St. Lawrence ($r = -0.77$, $n = 14$, $n_{eff} = 6$, $p < 0.05$, Figure 6.12). Therefore, the mid-1980s shift in migration timing coincided with older cod becoming more abundant and smaller in size, and also with cod's principal fish prey becoming more abundant on summer grounds.

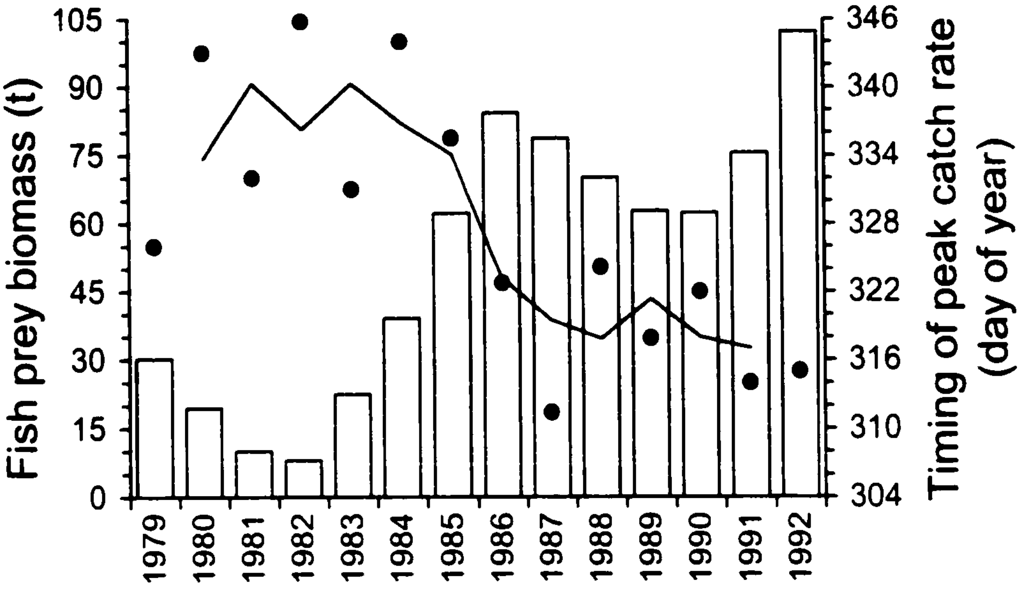

Figure 6.12. Timing of autumnal peaks in catch rates in the eastern Gulf (●, personal logbook series) in comparison to the combined abundance of herring and capelin (open bars). Herring abundance surveys started in 1978. The herring data (spring spawners) were taken from Claytor and LeBlanc (1999). The capelin data were taken from Frank et al. (1996).

DISCUSSION

Environmental cue

The timing of cod migrations varied little between 1970 and 1992, with standard deviations ranging from 8 d (spring) to 10 d (autumn). Although there are no analogies in the marine groundfish literature, similar but slightly lower (SD 2 to 6 d) interannual variation estimates have been reported for some salmonid (Jonsson and Ruud-Hansen 1985, Bohlin et al. 1993) and avian (Berthold 1996) species. In the latter group, the low interannual variation estimates were interpreted to indicate that photoperiod plays an important role in the mechanism cueing the seasonal migrations. For cod, however, the autumn migration has occurred progressively earlier since 1986, indicating that photoperiod could not have been an exclusive stimulus to migration. The reported trend in migration timing is consistent with the view of sentinel fishers, who commented that southern Gulf cod were migrating out of the Gulf noticeably earlier during the 1990s (Chouinard et al. 1999b). Moreover, Castonguay et al. (1999) suggested that a neighbouring stock, the northern Gulf of St. Lawrence cod, also performed the autumn migration earlier during the late 1980s and early 1990s. Therefore, it appears that the mid-1980s shift in the timing of the autumn migration was real and widespread inside the Gulf of St. Lawrence.

The trend in the autumn migration may explain changes in the distribution of cod observed in the annual demersal fish survey in the southern Gulf of St. Lawrence. This survey has always been conducted in September, a few weeks prior to the peak in catch rates in the west (upper Shediac Valley) for recent years. Swain (1995) observed that the proportion of cod survey biomass found in the eastern Gulf increased steadily since the beginning of the survey in 1971 and particularly since the mid-1980s. Recent observations (Poirier et al. 2000) indicate that this trend has continued. The changes in the distribution of cod in the survey, particularly the low abundance observed in the most westerly area near the Gaspé coast (Québec), is consistent with the trend for an earlier migration of cod found in this study.

It is unlikely that changing oxygen concentrations motivate southern Gulf cod to migrate seasonally. At the onset of the autumn migration, for instance, the oxygen levels in near bottom waters were increasing and well above those believed to induce physiological stress and avoidance behaviour in this species (< 5 mg ml^{-1}, Claireaux and Dutil 1992, D'Amours 1993, Claireaux et al. 1995b). The lack of association between oxygen cycling and onset of migration was not surprising, since animals migrating in response to low oxygen levels will most likely end up in an environment with improved oxygen concentrations. Cod, on the other hand, ultimately overwinter in deeper and hence less oxygenated waters. They also occupy comparatively warm waters in winter (Swain et al. 1998), whereas ectothermic animals facing hypoxia are expected to seek a colder environment to acquire physiological advantages, namely to reduce metabolic rate (Brett 1971) and to increase blood oxygen affinity (Riggs 1970, Alberts 1970, Weber et al. 1976, Boutilier et al. 1988).

I found little evidence that limiting food resources triggered migration events. On the one hand, cod initiated their autumn migration when food intake (assessed in terms of relative and absolute stomach content weight) was low. On the other hand, food intake continued to decline during the autumn migration and reached a minimum on winter grounds. Moreover, some individuals extended their summer residency inside the western Gulf: these cod were feeding extensively, suggesting that food was abundant in the Gulf throughout autumn. Therefore, it seems unlikely that a lack of food could have been the principal motivator for departure from summer grounds.

My temperature recorders indicated that the return of cod into the southern Gulf might coincide both temporally and spatially (eastern Gulf *v.* western Gulf) with the temperatures rising above a lower limit for cod distribution. This is consistent with the view that the warming of the coastal shelf environment in spring regulates the progression of the shoreward movements (Konstantinov 1965, Chen et al. 1994, Hutchings and Myers 1994, Colbourne et al. 1997). However, there is no evidence to date showing that the breakdown of the thermal barrier actually cues migratory behaviour. It is possible, for instance, that migratory restlessness appears some time before the breakdown of the thermal barrier, and that the latter event simply allows cod to pursue their migration into the southern Gulf. Certainly, my close examination of temperature changes in autumn suggests that temperature does not proximately cue migration. When cod initiated migration in the western Gulf (1995-1998), temperatures were rising but remained well within the median values occupied by this stock in September (0.3 to 7.5°C [age 5], Swain and Kramer 1995). More importantly, cod did not initiate migration at a specific temperature or following a particular temperature regime during the previous summer. This conclusion also held for the longer term (1970-1992), since the departure date in autumn was not correlated with thermal conditions in the CIL. My results thus oppose the suggestion that the departure date in autumn is dependent upon thermal conditions in the CIL during the previous summer (Castonguay et al. 1999, Dutil et al. 1999). Nevertheless, the CCF analysis indicated that the mid-1980s shift in migration timing lagged behind the declining temperatures by 1 to 2 years, which raises the possibility that the shift in timing was linked in some way to the cooling event. Below, I identify a potential physiological mechanism by which migration dates could have been changed by the cooling event.

Physiological cue

Seasonal patterns in food intake and energy storage have been described for both southern and northern Gulf cod (Schwalme and Chouinard 1999, Lambert and Dutil 1997b). In the present study, the trends in food intake and energy storage are compared with the onset of seasonal migrations. Cod were feeding little but the I_H peaked at the onset of the autumn migration between 1995 and 1997. These data suggest that cod moving out of the Gulf had just completed the seasonal process of lipid deposition, since the I_H is a very good indicator of the total lipid reserves in cod (Lambert and Dutil 1997a). By contrast, in spring, food intake and I_H values suggest that cod entering the Gulf had recently begun the task of re-building lipid reserves through intense feeding.

In theory, there are a few underlying mechanisms that could link migration onset to the above patterns in food intake and energy cycling. For instance, my results are consistent with laboratory evidence that internal lipid stores have a negative effect on appetite (Metcalfe and Thorpe 1992, Jobling and Miglavs 1993, Simpson et al. 1996). Since peak lipid deposition (i.e. peak I_H) in cod coincided with the onset of the autumn migration, it could be that a physiological suppression of appetite induces cod to leave summer feeding grounds. Likewise, because appetite is regained when lipid stores are depleted (Metcalfe and Thorpe 1992, Jobling and Miglavs 1993, Simpson et al. 1996), lipid depletion in spring could motivate cod to return to their feeding grounds simply by enhancing appetite. Previous laboratory work has also shown that low ration or starved fish (cod included) select colder temperatures compared to well-fed individuals (Javaid and Anderson 1967, Stuntz and Magnuson 1976, Mac 1985, Morgan 1993, Despatie et al. in press). In keeping with these reports, it may be that cod migrate into the Gulf in response to a shift in thermal preferendum in spring. Temperature recorders indicated that Gulf waters in spring, especially those in the western Gulf, tend to be colder than those in the Cabot Strait area. By migrating into the western Gulf, the starved fish would benefit from low-metabolic costs at low temperature (see Swain and Kramer 1995, Swain 1999, Despatie et al. in press). In autumn, peak energy stores may alter the thermal preferendum and cod may favour warm slope waters. For cod with sufficient energy stores, moving into warm waters might enhance gonadal development (see Hutchings and Myers 1994). Therefore, the onset of migration could be linked to food intake and energy stores patterns by at least two different physiological pathways (appetite and thermal preferendum).

It is interesting that a small fraction of the population extended their stay in the western Gulf until at least late October. These fish had significantly lower energy stores (I_H) than the bulk of the population that departed the southern Gulf in early October. Their 'decision' not to engage in migration may have been related to a poor feeding history, and thus to a sustained appetite, or to a continued preference for cold waters. The first interpretation is consistent with the fact that fish remaining on summer grounds in late October were feeding intensively. The second interpretation is consistent with our temperature recorders showing that near bottom waters in the western Gulf remained relatively (compared to slope waters) cold in autumn.

The energy stores model presented above predicts that the timing of the autumn migration will be inversely correlated with the availability of food resources on summer grounds, and more specifically with the availability of fish prey since they represent an important source of lipids (Black and Love 1986). I found a negative correlation between timing and the combined abundance of herring and capelin between 1979 and 1992 ($r = -0.77$, $p < 0.01$). Note also that fish prey became more prominent in the diet of southern Gulf cod in recent years: in 1980, for example, the proportion by weight of fish in the diet of large cod was 28%; in 1992, however, this figure increased to 74%, the highest reported since the mid-1950s (Schwalme and Chouinard 1999). I also report that southern Gulf cod performed the autumn migration earlier as the older (ages 11+) individuals in the population became more abundant ($r = -0.66$ to -0.76) and grew less rapidly ($r = 0.71$ to 0.93). A link between migration timing and the number of older cod is plausible since Rose (1993) showed that larger (older) cod lead migrations. It is also possible that, owing to their smaller body size and presumably lower metabolic requirements (i.e. food requirements) since 1986, cod feeding inside the Gulf reached the autumnal peak in energy stores earlier than normal, thereby precipitating an earlier departure. The rise in the number of older cod may have enhanced the effect, since these individuals are more experienced in capturing fish prey. In this model, the low metabolic requirements and rich diets would have advanced migration dates by accelerating the accumulation of fat in summer. If this interpretation is accurate, it could be argued that the widespread cooling of water masses (Gilbert and Pettigrew 1997) in the 1980s contributed to the earlier migration dates, since the cooling event likely reduced growth rates and widened the distribution of prey such as capelin (see Frank et al. 1996, Schwalme and Chouinard 1999).

This study has shown that the inter-year variability in the timing of the spring and autumn migrations was relatively low for southern Gulf cod, which raises the possibility that photoperiod was somehow involved in the control mechanism of migration. However, since cod initiated the autumn migration progressively earlier starting in the mid-1980s, it seems unlikely that photoperiod was an exclusive stimulus to migration. A widespread cooling of near bottom waters preceded the mid-1980s shift in migration timing by 1 to 2 years, suggesting that the mechanism triggering migration was linked to environmental temperature. It is likely that the cooling of water masses lowered the metabolic requirements of cod. As a working hypothesis, I propose that low metabolic requirements and high fish prey abundance led to a rapid build-up of energy reserves over summer grounds, and thus to an earlier physiological readiness to engage in the autumn migration.

CHAPTER SEVEN

Synthesis

The main objective of this thesis has been to identify the endocrinological and environmental factors controlling the seasonal migrations of Atlantic cod. Each chapter has provided new data on this topic. In the following synthesis, I will briefly review these data, and propose a plausible underlying mechanism for the control of cod migrations.

ENDOCRINE HORMONES

Gonadal Hormones

Since the migrations of southern Gulf cod are essentially spawning and post-spawning migrations, I examined whether gonadal hormones were correlated with migratory behaviour (Chapter 3). Testosterone cycling in southern Gulf cod showed no consistent pattern in relation to the seasonal migrations. Estradiol-17ß (E_2) increased to maximum levels during the spring migration, but it was unclear as to how the hormone could have been implicated in the control of migration; unlike androgenic steroids, E_2 has no documented effect on swimming activity. Moreover, E_2 was found in negligible concentrations in males, implying that males would not undergo endocrinological changes favouring migration, and that males would simply follow females on their migratory journey. Such a mechanism is inconsistent with recent observations suggesting that males reach coastal spawning grounds first (Lawson and Rose 2000). Clearly, a link between gonadal hormones and migratory behaviour seems doubtful for cod.

Thyroid Hormones

This thesis provided evidence of remarkable thyroid hormone cycles in cod (Chapter 2). Moreover, the cycles were synchronized with both the autumn and spring migrations. Mean T_4 and T_3 levels were significantly higher at the onset of the autumn migration than during the summer residency, and interestingly remained elevated until cod reached wintering grounds (Chapter 3). There were also indications that high T_4 individuals migrated out of the Gulf faster than their low T_4 counterparts in autumn. During the return migration in spring, there was a tendency to release a larger fraction of the metabolically potent T_3 hormone from binding proteins (Chapter 4). Together these data suggest that the thyroidal and peripheral systems are somehow involved in the control of seasonal migrations. It seems likely that thyroid hormones at least facilitated the energy-demanding seasonal migrations by exerting a stimulatory effect upon locomotion (Woodhead 1970, Castonguay and Cyr 1998) and the general metabolism.

Woodhead (1975) conceptualised that thyroid hormones motivate cod to turn and swim against the prevailing ocean current, and thus in effect proximately trigger migration. However, field data in the current thesis were inconsistent with this model. First, thyroid hormone levels during the upstream migration were either equal to or lower than those recorded during the downstream migration (Chapter 3). Second, southern Gulf cod did not initiate migration at a specific thyroid activity level over a four-year period. Third, it seems that the thyroid is invariably activated at the September equinox, whereas historic catch rates indicated that cod moved out of the southern Gulf in late autumn during the 1970s (Chapter 6). Lastly, the equinoxial upsurge in thyroidal activity might regulate the timing of gonadal development (Chapter 4). All these observations shed doubt on the premise that seasonal changes in thyroidal activity are concerned primarily with the onset of migratory behaviour (Woodhead 1975). Considering recent technological advances in open water telemetry, future work might involve tracking thyroid-manipulated subjects in their natural environment. As a step forward in such an experiment, this thesis has demonstrated that sustained (1 month) experimental hyper- and hypothyroidism is feasible in wild cod by osmotic pump delivery of thyroid or anti-thyroid agents (Chapter 5).

ENVIRONMENTAL FACTORS

Photoperiod

My analysis of cod catch rates (Chapter 6) suggests that the timing of seasonal migrations varied little between 1970 and 1999, with standard deviations ranging from 8 (spring) to 10 (autumn) d. Similar interannual variation estimates have been reported for some avian species (Berthold 1996), and these were interpreted to indicate that photoperiod plays an important role in the mechanism triggering the seasonal migrations. For southern Gulf cod, however, the autumn migration has occurred progressively earlier since 1986: the bulk of the population transited through the eastern Gulf near November 30 between 1970 and 1985, November 14 between 1986 and 1992, and November 1 between 1995 and 1999. In keeping with a photoperiod-trigger, it could be hypothesized that the mid-1980s shift in migration timing was due to genetic selection for early autumn migrants, i.e. selection for the individuals that were programmed to respond to early autumn photoperiod conditions. In this case, the selection process would have been caused by an intense and sustained fishing effort directed towards the late autumn migrants. However, there is currently no evidence of such selective fishing or of inherited control mechanisms of migration for cod. Therefore, the present information suggests that photoperiod does not function as a primary or exclusive trigger for departure. This conclusion appears to make sense from an evolutionary perspective: a flexible trigger mechanism would insure that migratory movements are initiated at the appropriate times within environmental and physiological cycles, so that the evolutionary benefits of migration outweigh the risks.

Oxygen and Temperature

At the onset of the autumn migration, dissolved oxygen concentrations were above levels known to induce avoidance behaviour (Chapter 6). Bottom water temperatures were rising but remained well within the median values occupied by this stock during the annual groundfish survey. Moreover, over a four-year period, cod did not initiate the autumn migration at a specific temperature or following a particular temperature regime during the previous summer. In spring, before the return of cod, ocean bottom temperature recorders identified a thermal barrier (< -0.5°C) over the main summer grounds. The progressive breakdown of this barrier was consistent with migration routes and historical migration dates, suggesting that the warming of the shelf environment regulates the progression of shoreward movements. Whether the warming event might also cue migratory behaviour *per se* could not be answered. From a general viewpoint, it seems unlikely that the underlying mechanism triggering migratory behaviour in cod would be sensitive to environmental temperatures in spring but not in autumn.

Food Resources

The mid-1980s shift in migration timing lagged behind a widespread cooling of water masses by 1 to 2 years (Chapter 6), suggesting that the shift in timing was linked in some way to the cooling waters. The cooling event may have widened the distribution of prey such as capelin into the southern Gulf of St. Lawrence (Frank et al. 1996, Carscadden et al. 2001). Certainly, there is evidence that capelin and herring prey were historically prominent in the diet of southern Gulf during the early 1990s (Schwalme and Chouinard 1999). In view of these data, I examined the possibility that a high consumption of fish prey somehow resulted in an early readiness to engage in migration. Support for this mechanism was provided by an inverse correlation ($r = -0.77$) between autumn migration timing and the abundance of herring and capelin on summer grounds. Additional support was provided by the I_H, which indicated cod moved out of the summer feeding grounds immediately after completing a seasonal process of energy (lipid) deposition. It thus seems possible that the rich diets in the 1990s accelerated the seasonal fat storage process, and in doing so, advanced departures from feeding grounds.

Theoretically, the fat storage cycle in cod could trigger migration by a least two different physiological pathways, namely by affecting appetite (Metcalfe and Thorpe 1992, Jobling and Miglavs 1993, Simpson et al. 1996) and temperature preferences (Javaid and Anderson 1967, Stuntz and Magnuson 1976, Mac 1985, Morgan 1993, Despatie et al. in press). More precisely, peak energy stores may cue departure from feeding grounds by suppressing appetite and/or by shifting the temperature preferendum towards warm slope water. Likewise, low energy reserves in spring may motivate cod to return onto feeding grounds by enhancing appetite and/or by shifting the thermal preferendum towards the low-metabolic costs shelf temperatures. This interpretation is consistent with the observation that southern Gulf cod migrated back onto feeding grounds when the task of re-building energy reserves had begun through intense feeding (Chapter 6). Therefore, energy cycling in cod represents a possible factor influencing the timing of both the autumn and spring migrations.

It is also noteworthy that southern Gulf cod performed the autumn migration earlier as the older (ages 11+) individuals in the population became more abundant (r = -0.66 to -0.76) and grew less rapidly (r = 0.71 to 0.93). The fact that these correlations were significant only for the older fraction of the population is in broad agreement with echosounding data showing that the larger cod lead migratory aggregations (Rose 1993), and thus presumably determine migration schedules for the entire population. Exactly how the older fish may have advanced migration dates is unclear, although some explanations are consistent with the above energy store model. For instance, it may be that the rise in the number of older cod enhanced the suspected effects of abundant capelin and herring, considering that the more experienced cod probably have more success in capturing fish prey. Also, owing to their smaller body size and presumably lower metabolic requirements since 1986, cod feeding inside the Gulf may have reached the autumnal peak in energy stores earlier than normal, thereby precipitating an earlier departure. This statement may be valid also for the northern Gulf of St. Lawrence cod (for growth rates, see Dutil et al. 1999; for migration, see Castonguay et al. 1999).

To sum up, I propose that the mechanism triggering cod migrations is sensitive to the annual cycle of energy deposition / utilization. Energy stores, and more precisely lipid reserves, may conceivably regulate migration timing by affecting appetite and/or the temperature preferendum. In recent years, it seems likely that a reduction in metabolic requirements (growth rates), coupled to an increase in the consumption of fish prey, led to a rapid build-up of lipid reserves on summer grounds. These conditions may have resulted in an earlier readiness to engage in the overwintering migration.

IMPLICATIONS OF EARLY DEPARTURES FROM FEEDING GROUNDS

Stock Productivity

The evolutionary implications of earlier departures from feeding grounds in recent years are not clearly understood. An obvious advantage for leaving summer grounds as quickly as possible could be the enhancement of gonadal development in warm slope waters (see Hutchings and Myers 1994). On the other hand, it does not appear that cod are capable of returning into the Gulf sooner the following spring, given the presence of ice (Fréchet 1990) and a thermal barrier described earlier. It seems that an early arrival on winter grounds increases the time spent where food resources are possibly scarce or inaccessible due to vertical separation of cod and its fish prey (Schwalme and Chouinard 1999). An open question is whether the prolonged utilization of energy reserves over winter grounds had a negative impact on stock productivity, given that winter starvation is thought to result in negative growth (Dutil and Lambert 2000), reduced reproductive potential (Kjesbu et al. 1991, Lambert and Dutil 2000) and increased risk of mortality (Dutil and Lambert 2000). Certainly, such an effect is difficult to imagine considering the above model for migration onset: the idea that an abundance of food supply on summer grounds can ultimately be detrimental to the stock raises an evolutionary paradox. A more plausible explanation is that the lipid-rich diets since the mid-1980s favoured an extended period of residence in the winter area. Lipids are by far the most efficient vehicles for storing chemical energy, and it seems that avian species at least accumulate more lipids than are needed to cover the cost of migration related activities (Berthold 1996).

Stock Management

In recent years, the migration from west to east within the southern Gulf was initiated in early October (Chapter 6), and thus close in timing to the annual (September) demersal fish survey. Swain (1995) observed that the proportion of cod survey biomass found in the eastern part of the southern Gulf increased since the late 1980s, consistent with the migration route and the trend for earlier migration timing presented in this thesis. It is possible that the increasing proportion of cod biomass found in the eastern Gulf was due in part to the advancement of migration schedules.

Fishery managers have long recognized that a substantial portion of the southern Gulf cod population reaches the Cabot Strait, and starts mixing with other populations in the area (Campana et al. 1999, Swain et al. 2001), in November. Despite the earlier onset of the autumn migration in recent years, I found no indications that the southern Gulf cod arrived into the Cabot Strait before November, consistent with current stock management. However, this conclusion may not hold with further advancement of autumn migration schedules. In order to provide a rigorous conservation strategy, the timing of the seasonal migrations should be monitored.

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