INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON OVARIAN DEVELOPMENT AND SPAWNING OF SMALLMOUTH BASS (*Micropterus dolomieui*)

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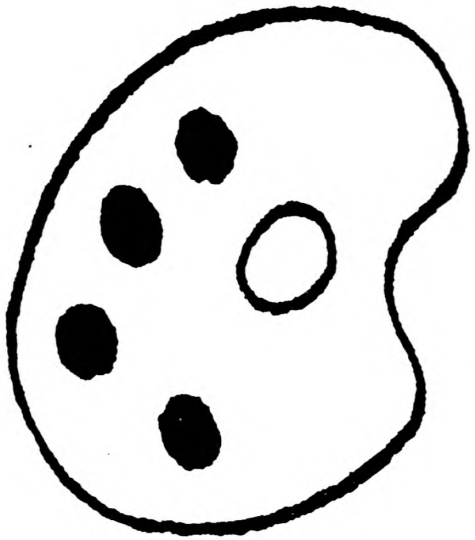
NUMEROUS ORIGINALS IN COLOUR



DEDICATED TO "BIG MAMA"

a thirteen year old wild smallmouth bass, first *in vivo* sampled in a natural environment and slowly adapted to laboratory conditions who died during the winter of 1993 at the age of nineteen.

PREFACE

The study was conducted in the facilities of Aquaresearch Ltd, the only commercial grower of smallmouth bass in Quebec. The unique installations included rearing systems with water recirculation, a laboratory for physico-chemical analyses of water and fish manipulation and a research pond. Specific agreements were made with the Collège de Sherbrooke to have access to the equipment for the histological sectioning and, with the Centre Hospitalier de l'Université de Sherbrooke (CHUS) to analyze specific blood parameters. Fish used in the experiment were from wild populations or first generation raised in captivity. Fertilization, in smallmouth bass, has not been artificially realized yet; so artificial nests were used to simulate natural spawning grounds.

Work on larval rearing of smallmouth bass started, at Aquaresearch Ltd, in 1983 and was followed two years later by the environmental control of spawning. Previous studies are presented in the Appendices.

This dissertation starts by defining the natural and controlled frequency and timing of spawning, then goes on with the morphology and histology of the ovary, followed by the changes in gonado-somatic indices, condition factors and blood parameters as influenced by constant and seasonal conditions. The last section deals with the effects of temperature and photoperiod on aspects of the reproductive rhythm of smallmouth bass.

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Thesis submitted for the degree of Doctor of Philosophy

by Marie-Claude Cantin, Institute of Aquaculture, University of Stirling.

Abstract

The reproductive cycle of smallmouth bass (*Micropterus dolomieui*) from the 45°14'N latitude and 71°58'W longitude has been investigated with regards to constant temperature-photoperiod regimes, seasonal and protracted cycles over two years. The morphology and the histology of the ovary, the changes in plasmatic components and the uni-multi-spawning capacity were followed to verify the influence of temperature and photoperiod regimes, and the plasticity of smallmouth bass reported as eurytopic. Results showed individual variability of ovarian development partially related to the desynchronizing effects of constant conditions and a group-synchronous ovary with a possible asynchronous ovarian development under constant conditions. A positive correlation between plasma calcium and the presence of vitellogenic oocytes was also noted. Plasma protein, calcium and oestradiol-17β levels increased with vitellogenic activity. The presence of atresia was associated with the maintenance of vitellogenic activity over long periods particularly under constant winter conditions. Oocytes did not mature under a second year of constant summer conditions. Smallmouth bass showed a capacity of spawning one to four times a year at 15-30 day intervals. Fecundity and nest productivity were highly variable (500-18,000 oocytes and 0-8,500 eggs released, respectively) but within the range of previous studies. The study delineated the proximate and ultimate controls of temperature on ovarian maturation and spawning regardless of the photoperiod regime. Photoperiod was not shown to have any direct effect on the reproductive cycle. Controlled temperature-photoperiod conditions can induce spawning in any month of the year. Temperature was the decisive factor in the ovarian development and the spawning of smallmouth bass, as it has been shown to be for other teleosts. Sequential sampling of fish demonstrated individual and seasonal variations of ovarian development and synchronization.

Furthermore, fish can modulate their spawning tactics based on environmental conditions.

ACKNOWLEDGEMENTS

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Finally I want to express my warmest thanks for my husband, Karl, who had to live with a "student" during the six years it took to complete this study and whose moral support and statistical knowledge have been greatly appreciated.

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LIST OF FISH SPECIES

English Names	Latin Names (Genus & Species)	Family Names
African catfish	*Clarias gariepinus*	Clariidae
Amazonian characin	*Brycon erythropterus*	Characinidae
anchovy	*Engraulis mordax*	Engraulidae
Arctic charr	*Salvelinus alpinus* L.	Salmonidae
Asian seabass	*Lates calcarifer*	Centropomidae
Atlantic halibut	*Hippoglossus hippoglossus*	Pleuronectidae
Atlantic herring	*Clupea harengus* L.	Clupeidae
Atlantic salmon	*Salmo salar*	Salmonidae
barbel	*Barbus barbus*	Cyprinidae
bluegill	*Lepomis macrochirus*	Centrarchidae
brook stickleback	*Eucalia inconstans*	Gasterosteidae
brook trout	*Salvelinus fontinalis*	Salmonidae
brown bullhead	*Ictalurus nebulosus*	Ictaluridae
brown trout	*Salmo trutta*	Salmonidae
carp	*Cyprinus carpio*	Cyprinidae
catfish	*Clarias macrocephalus* G.	Clariiidae
channel catfish	*Ictalurus punctatus*	Ictaluridae
Chinese catfish	*Clarias fuscus* L.	Clariidae
chub	*Leuciscus cephalus*	Cyprinidae
cod	*Gadus morhua*	Gadidae
Coho salmon	*Onchorynchus kisutch*	Salmonidae
dab	*Limanda limanda*	Pleuronectidae
dace	*Leuciscus leuciscus*	Cyprinidae
Dover sole	*Microstomus pacificus*	Pleuronectidae
eel	*Anguilla anguilla*	Anguillidae
	Encrasicholina heteroloba	Engraulidae
flounder	*Platichthys flesus* L.	Pleuronectidae
fourspine stickleback	*Apeltes quadracus*	Gasterosteidae

gilthead seabream	*Sparus aurata*	Sparidae
goby	*Gillichthys mirabilis*	Gobiidae
golden perch	*Macquaria ambigua* R.	Percichthyidae
golden shiner	*Notemigonus crysoleucas*	Cyprinidae
goldfish	*Carassius auratus*	Cyprinidae
green sunfish	*Lepomis cyanellus*	Centrarchidae
grey mullet (mullet)	*Mugil cephalus*	Mugilidae
gudgeon	*Gobio gobio* L.	Cyprinidae
honmoroko	*Gnathopogon caerulescens*	Cyprinidae
horse mackerel	*Trachurus trachurus* L.	Carangidae
Indian catfish (singi fish)	*Heteropneustes fossilis*	Clariidae
Indian mackerel	*Rastrelliger kanagurta*	Scombridae
killifish	*Fundulus grandis*	Cyprinodontidae
	Labeo frenatus	Cyprinidae
largemouth bass	*Micropterus salmoides*	Centrarchidae
longjaw goby	*Gillichthys mirabilis*	Gobiidae
marsh killifish	*Fundulus confluentus*	Cyprinodontidae
milkfish	*Chanos chanos*	Chanidae
	Mirogrex terrae-sanctae	Cyprinidae
mummichog	*Fundulus heteroclitus*	Cyprinodontidae
orangemouth corvina	*Cynoscion xanthulus*	Sciaenidae
Pacific mackerel	*Scomber japonicus*	Scombridae
perch	*Perca fluviatilis*	Percidae
pike	*Esox lucius*	Esocidae
plaice	*Pleuronectes platessa*	Pleuronectidae
rabbitfish	*Signatus guttatus*	Siganidae
rainbow trout	*Onchorynchus mykiss*	Salmonidae
red drum	*Sciaenops ocellatus*	Sciaenidae
red-tailed shark	*Labeo bicolor*	Cyrpinidae
sand borer	*Sillago sihama*	Sillaginidae
seabass	*Dicentrarchus labrax*	Moronidae

silver perch	*Bidyanus bidyanus*	Teraponidae
smallmouth bass	*Micropterus dolomieui*	Centrarchidae
sole	*Solea solea*	Soleidae
speckled trout	*Salvelinus fontinalis*	Salmonidae
spotted seatrout	*Cynoscion nebulosus*	Sciaenidae
striped bass	*Morone saxatilis*	Percihthyidae
tench	*Tinca tinca*	Cyprinidae
threespine stickleback	*Gasterosteus aculeatus*	Gasterosteidae
tomcod	*Microgadus tomcod*	Gadidae
turbot	*Scophthalmus maximus*	Bothidae
white sucker	*Catostomus commersoni*	Catostomidae
whitefish	*Barbus andrewi*	Cyprinidae
winter flounder	*Pseudopleuronectes americanus*	Pleuronectidae
yellowtail snapper	*Ocyurus chrysurus*	Lutjanidae

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CHAPTER 1

GENERAL INTRODUCTION

In recent years, studies of the reproductive cycles of a variety of fish, other than salmonids, have dealt particularly with gonadal cycles (review by Fontaine, 1976; Hoar, 1969; Peter & Crim, 1979; Nagahama, 1983; de Vlaming, 1983; Selman & Wallace, 1986), with seasonal changes in plasma/serum components (Whitehead *et al.*, 1978a; Scott *et al.*, 1980; Crim & Idler, 1978; Norberg *et al.*, 1989; Wiener *et al.*, 1985; Sandstrom, 1989; Clark *et al.*, 1979; Wingfield & Grimm, 1977; Dickhoff *et al.*, 1989; de Vlaming *et al.*, 1984) and the importance of environmental cues in timing reproductive seasonality (reviewed by de Vlaming, 1972a; Crim, 1982; Lam, 1983; Bye, 1984; Munro *et al.*, 1990). Aquaculture and the development of techniques to control reproduction have directed research towards a better knowledge of reproductive seasonality. The control of ovarian development and spawning is an endogenous mechanism modulated by specific environmental and social cues recognized by the fish to allow survival of the species. Environmental cues, which have been studied, include monsoons (Lamba *et al.*, 1983), tides and lunar phases (Taylor, 1984; 1990), floods (Munro, 1990), specific food items and availability (Munro, 1990), salinity (de Vlaming, 1972a), water temperature (Bye, 1990) and photoperiod (Bromage *et al.*, 1990). However, it is temperature and/or photoperiod that have been the most extensively studied because of their importance in the initiation of gonadal recrudescence and regression in many teleosts (Peter, 1981).

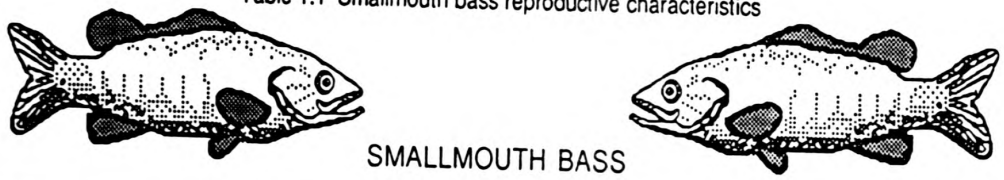
This research assessed the ovarian development and spawning of smallmouth bass in relation to constant, extended seasonal and natural temperature-photoperiod regimes. The investigations' techniques included histological sectioning, *in vivo* sampling of oocytes, and monitoring of fluctuations in gonado-somatic index (GSI), condition factors and plasma calcium, total protein and oestradiol concentrations. All parameters examined fluctuated with the reproductive stage of the teleosts and have often been used as indicators of the status of the ovarian cycle (West, 1990).

Constant photoperiod and/or temperature conditions have often been used to study endogenous rhythms particularly the circannual rhythm of gonadal maturation in rainbow trout (Duston & Bromage, 1986, 1987, 1988, 1991), hormonal regulation (Gern *et al.*, 1992) and lunar and semi-lunar cycles of reproduction (Leatherland *et al.*, 1992) to mention a few. There has been some criticism about constant conditions used to illustrate "normal rhythms" somehow related to the definition of a normal rhythm under captive or laboratory conditions. However, constant conditions should be considered as one tool used to illustrate the variation of the endogenous rhythm independently of seasonal variations of cues (photoperiod and temperature).

The smallmouth bass is a highly prized game fish in North America. It is a perciform member of the centrarchid family, found from the southern USA (34°N lat.) to southern Canada (45°N lat.) (MacCrimmon & Robbins, 1975). There have been hundreds of introductions in many different countries but only South Africa, Vietnam and Hawaii have maintained viable populations (Robbins & MacCrimmon, 1974). The reason for the success rearing of these populations has been attributed to the presence of appropriate temperatures (Robbins & MacCrimmon, 1974).

The genus *Micropterus* stands for small-finned fishes. The Centrarchidae are generally coloured fishes, with big eyes. They are spring and/or summer spawners with often, a protracted spawning period. The males generally build a nest and guard it after spawning has occurred. Field observations of females have shown that they can spawn in more than one nest, on more than one occasion and that there is a wide variability in the number of eggs released per nesting and per season (Fig. 1.1). De Vlaming (1983) reported that species in this family had group-synchronous oocyte development, one of the most common patterns with two or more clutches of oocytes present prior to or during the spawning season. Finally, the members of the centrarchid family apparently share different spawning strategies with multi or uni-spawning over a short or long period of time. Smallmouth bass have been reported as quite eurytopic (able to tolerate a wide range of habitats) and adaptable to a variety of situations (Jackson, 1991).

Table 1.1 Smallmouth bass reproductive characteristics



SMALLMOUTH BASS

REPRODUCTIVE CHARACTERISTICS	MALES	FEMALES	BOTH SEXES
Sexual Maturity •wild •age •size •controlled conditions •age •size	3-5 yrs (Scott & Crossman, 1974) 19.4 cm (Stone *et al.*,1954) 11 months 20 cm (Ehrlich *et al.*, 1986)	4-6 yrs (Scott & Crossman, 1974) 25 cm (Stone *et al.*,1954) 11 months 22.1 cm (Ehrlich *et al.*, 1986)	
# Fertile Years		5-12 (Coble, 1975)	
Life Span •wild •captivity			15 years (Coble, 1975) 20+ years
Spawning Temp.			18°C
Spawning Photoperiod			14 hours and increasing (Cantin & Bromage, 1991)

cont

Table 1.1 Smallmouth bass reproductive characteristics (cont.)

Spawning Time •wild •controlled			May - June Jan.- mid July, October up to 8 months of the year (Cantin, 1987a,b; Cantin & Bromage, 1991)
Number of Spawnings			1 (Surber, 1943) 3 (Beeman, 1924) 8 (Inslee, 1975) 1-4 (Cantin, 1988; Cantin & Bromage, 1991)
Fecundity (# ripe eggs)		5,000 - 14,000 (Scott & Crossman, 1974) 4,500 (Clady, 1970) 2,000 - 15,000 (Coble, 1974)	
Fertilization Rate			0-100%

Table 1.1 Smallmouth bass reproductive characteristics (cont.)

Nest Productivity (# eggs/nest) •wild •controlled			1000 - 5,000 (Latta, 1963) 92 - 11,329 (Langlois,1935) 1,241 - 14,120 5,238 (Inslee, 1975)
Hatching Success (# larvae/nest)			7,000 (Scott & Crossman, 1974) 2,526 (Langlois, 1930) 2,253 (Inslee, 1975)
Behaviour: prespawning spawning postspawning outside spawning season	prepare nest guard eggs & larvae	stay in the dark, at cooler temperature	spatial requirement (Cantin, 1987b) together

The propagation of smallmouth bass started as early as 1877 in Ohio (Langlois, 1935), but there is limited information on their ovarian development and their response to controlled conditions. Field studies of smallmouth bass life history included growth studies (Fraser, 1955; Turner & MacCrimmon, 1970; King *et al.*, 1991), natural history and behaviour (Doan, 1940; Coble, 1975; Miller, 1975), movement and habitat preference (Sechnick & Carline, 1986; Todd & Rabeni, 1989), reproduction and rearing (Beeman, 1924; Tester, 1932; Langlois, 1935; Surber, 1943; Schneider, 1971; Inslee, 1975; Neves, 1975; Reynolds & O'Bara, 1991); blood composition (Shell, 1961), population dynamics (Rideout & Oatis, 1975; Fajen, 1975) and the effects of temperature on different aspects of bass natural history (Coutant, 1975). However, it is only recently that laboratory studies have begun to broaden our knowledge of smallmouth bass in terms of larval nutrition and rearing (Ehrlich *et al.*, 1986, 1989) and controlled reproduction (Cantin, 1987a, b, c, 1988; Cantin & Bromage, 1991).

The natural reproductive cycle of smallmouth bass has been extensively described (review by Coble, 1975) with particular attention to spawning habitat and male behaviour (Reynolds & O'Bara, 1991; Hoff, 1991; Shuter & Ridgway, 1991). Up to now, field studies on spawning strategy have been mainly male oriented, and the limited information on female reproductive characteristics has sometimes been contradictory (Fig. 1.1). Males build their nest on gravel/sandy protected shores whilst females stay in deeper waters until ready to spawn. This pre-spawning behaviour starts when water temperatures are about 14-15C° and is followed by pre-nuptial activity (17-20°C) where the male turns around the nest in a circle moving slowly toward a female, inviting her to approach the nest and touching her sides. During nuptial activity, male and female turn around the nest together in a sort of dance as the female releases batches of 30-50 eggs. The process of spawning may last several hours before completion (Schneider, 1971). The interval between emissions varies from a few seconds (Reighard, 1905) to three minutes (James, 1930). The range of temperatures for optimal spawning up to the development of swim-up stage has been reported to be 15°C to 25°C (Coutant, 1975). The same nest can receive the eggs of different

females. Eggs have been most often found in the morning indicating that spawning had occurred during the night.

The eggs are demersal and measure between 1.2 mm (Scott & Crossman, 1974) to 2.8 mm (Reighard, 1905) and are on average 2.5 mm (Bower, 1897). They are guarded and ventilated by the male during incubation (2-10 days), the post-hatching period (5-15 days) and post-larval development for a total duration of a month or more (Coble, 1975). At hatching, the transparent bass hide in the gravel for 5-15 days after which they swim-up from the nest with the particular black pigmentation that has attibuted to them being called blackbass. At this stage, they form a school and start slowly to learn about their environment moving progressively away from the nest whilst they still remain under the paternal care.

A second nesting may take place after a period of adverse weather conditions (Coble, 1975). However, there has been some contradictions as to whether the same female or different females were involved in the second spawning. Beeman (1924) stated that a female can spawn three times per season whereas Surber (1943) stated that a female spawns out completely and does not produce eggs at intervals. Inslee (1975) reported eight spawnings a season. Sanderson (1958) observed different degrees of ovarian development from the same pool of smallmouth bass and thought it was indicative of different spawning time.

Natural spawning in captivity has been achieved for a number of fish, including striped bass (Curry Woods III *et al.*, 1990), whitefish (Bok & Immelman, 1989), tomcod (Watson, 1987), sole (Devauchelle *et al.*, 1987), turbot (Bromley *et al.*, 1986; Devauchelle *et al.*, 1988; McEvoy, 1989), orangemouth corvina (Prentice *et al.*, 1989), smallmouth bass (Anonymous, 1984; Cantin, 1988; Cantin & Bromage, 1991). Environmentally induced and/or controlled spawning has been developed for a variety of fish for practical reasons. These include, milkfish (Marte *et al.*, 1988), red drum (Arnold, 1988), seabass (Devauchelle & Coves, 1988; Carrillo *et al.*, 1989), turbot (Fores *et al.*, 1988), sand borer (Cheng-Seng & Hirano, 1985), cod (Bye & Htun-Han,

1979), gilthead sea bream (Bye, 1987), brook trout (Allison, 1951; Henderson, 1963); Coho salmon (MacQuarrie *et al.*, 1978), rainbow trout (Whitehead *et al.*, 1978b; Bromage *et al.*, 1984; Bromage & Duston, 1986), review on salmonids (Billard, 1989), largemouth bass (Brauhn *et al.*, 1972; Carlson, 1973; Carlson & Hale, 1972; Jackson, 1979), smallmouth bass (Cantin, 1988), Chinese catfish (Young *et al.*, 1989), barbel and chub (Poncin, 1988; 1989), tench (Poncin *et al.*, 1987), goldfish (Woynarovich & Horvath, 1981) and Pacific mackerel (Leong, 1977). Use of hormones in inducing maturation and/or ovulation and/or spawning (review by Zohar, 1988) has also been extensively studied particularly in fish that do not spawn easily in captivity like carp (review Pullin, 1986), yellowtail snapper (Soletchnik *et al.*, 1989), Asian seabass and rabbitfish (Harvey *et al.*, 1985), Amazonian characin (Eckmann, 1984), silver perch (Rowland, 1984), golden perch (Rowland, 1983), grey mullet (Kuo *et al.*, 1974), spotted seatrout, red drum, orangemouth corvina (Thomas & Boyd, 1988), milkfish (Marte *et al.*, 1988), *Labeo bicolor* and *Labeo frenatus* (Poncin *et al.*, 1988), striped bass (Curry Woods III *et al.*, 1992), Pacific mackerel (Leong, 1977), rainbow and brown trout (Billard *et al.*, 1984) or to improve spawning synchronization as in Atlantic salmon (Crim & Glebe, 1984).

While ovarian development has been studied and defined for a variety of fish (reviewed by Wallace & Selman, 1981; de Vlaming, 1983), description of ovarian development in smallmouth bass has been limited to the mature stages observed during the spawning period (Surber, 1943; Clady, 1970). Fecundity, expressed as the number of eggs released at spawning, has been estimated for a variety of fish (Vladykov, 1956; Urban, 1988; DuBois *et al.*, 1989; Bromage *et al.*, 1992a; Hunter *et al.*, 1992). However, it should be closely related to ovarian development and the reproductive strategy of the fish. Many aspects of atresia in fish have been discussed in the literature; these include description and dynamics (Khoo, 1975; de Vlaming *et al.*, 1984), importance (Bromage & Cumaranatunga, 1987), seasonality (Beach, 1959; Hunter & Macewiczs, 1985), influence on subsequent oogenesis (Trippel & Harvey, 1990), impact on fecundity (Treasurer, 1990) or the role of the postovulatory *corpora lutea* on the reproductive behaviour of fish (Lam *et al.*, 1978). A wide variety of teleosts have been studied (see

review by Pickford & Atz, 1957; Ball, 1960; Dodd, 1960; Ingram, 1962). However, atresia in smallmouth bass has not yet been described.

GSI has been used in conjunction with oocyte diameter and/or ovarian histology and/or blood parameters in many fish (Lambert *et al.*, 1978; Dindo & MacGregor, 1981; vanBohemen *et al.*, 1981; Burke *et al.*, 1984; Riazi & Frémont, 1988; Rosenblum *et al.*, 1987; Ouchi *et al.*, 1988, 1989; Everson *et al.*, 1989; Singh & Singh, 1990; Bradford & Stephenson, 1992) to assess ovarian growth and maturation. Gonadal weights were related to spawning activity in smallmouth bass (Reynolds, 1965), and in assessing the influence of photoperiod and/or temperature on gonadal cycles in green sunfish (Kaya & Hasler, 1972) and goldfish (Gillet *et al.*, 1978). Gonadal weights were also associated with brook trout fecundity (Vladykov, 1956). In temperate species, the GSI fluctuates seasonally peaking at the time of spawning and has been used as an indicator of the different phases of gamete maturation. Wilk *et al.* (1990) showed these annual cycles of gonado-somatic indices for fourteen marine fish species and associated it with spawning activity.

The condition factor, frequently used in fisheries, is an indicator of well-being of the fish based on an isometric growth pattern. Smallmouth bass from Des Moines River (Reynolds, 1965) followed the "cube law" of growth. However, if growth is allometric e.g. when shape changes as fish grow (LeCren, 1951), the relative condition factor (Kn) compensates for the morphometric variations, and has been used to compare males and females perch collected in different seasons within one population (LeCren, 1951), to compare herring and plaice larvae during growth and starvation (Ehrlich *et al.*, 1976) and to follow the growth of smallmouth bass from Ontario lakes (Tester, 1932). Condition factors associated with gonad weight have been used to assess seasonal changes related to reproductive activity in the perch (LeCren, 1951) and in the gudgeon (Kestemont, 1990).

More recently, plasma components have been monitored in relation to normal and/or altered reproductive cycles in many teleosts: rainbow trout (Whitehead *et al.*, 1978a;

Lambert *et al.*, 1978; Scott *et al.*, 1980; Scott & Sumpter, 1983a,b; Copeland *et al.*, 1986; Papoutsoglou, 1986; Cyr *et al.*, 1987), brown trout (Crim & Idler, 1978; Norberg *et al.*, 1989), goldfish (Oguri & Takada, 1967), white sucker (Wiener *et al.*, 1985), perch (Sandstrom, 1989), largemouth bass (Clark *et al.*, 1979), plaice (Wingfield & Grimm, 1977), Atlantic salmon (Dickhoff *et al.*, 1989), Atlantic halibut (Crim & Methven, 1989), cottidae (de Vlaming *et al.*, 1984) and cyprinidae (Aida, 1988). Only one study (Shell, 1961) has measured blood parameters in smallmouth bass. Studies of plasmatic components have included total plasma or serum proteins for a variety of fresh and saltwater fish (Deutsch & McShawn, 1949; Shell, 1961; Booke, 1964a, b; Ingram & Alexander, 1977; McKenzie & Paim, 1969; Alexander, 1977; Mulcahy, 1970; McCarthy *et al.*, 1973; Denton & Yousef, 1975; Hara *et al.*, 1980; de Vlaming *et al.*, 1980; Tinsley, 1985; Hunn & Greer, 1990); vitellogenin levels (reviewed by Ng & Idler, 1983; Ho, 1991) directly or through calcium concentrations (Elliott *et al.*, 1984) and seasonal changes in oestrogen levels (Wingfield & Grimm, 1977; Billard *et al.*, 1978; Crim & Idler, 1978; Dindo & MacGregor, 1981; Bromage *et al.*, 1982a; Burke *et al.*, 1984; MacGregor III *et al.*, 1985; Tam *et al.*, 1986; Pankhurst & Conroy, 1987; Ouchi *et al.*, 1988; Prat *et al.*, 1990).

It is well established that the liver of non-mammalian vertebrates is a target organ of oestrogen. In response to the steroid, many changes occur in the liver. The most noticeable change is the induction of vitellogenin synthesis, but there is also increased lipogenesis and lipid deposition, enhanced calcium and phosphate metabolism, suppression of production of albumin, induction of synthesis of very low density lipoproteins (VLDL), riboflavin carrier protein, transferrin and several other proteins (Ho, 1987; 1991). In most oestrogen target tissues, oestradiol-17β (E-2) is the most potent of the oestrogens in eliciting a biological response (Selcer & Leavitt, 1991). Many studies have verified the effects of E-2 injections on the control of ovarian development (eg, Bromage *et al.*, 1982a) and on oocyte development (Lessman & Habibi, 1987) confirming directly or indirectly that vitellogenin synthesis and secretion (egg yolk precursors) are controlled by oestrogens. The process of vitellogenesis in female teleosts involves the hepatic synthesis and subsequent ovarian accumulation of

yolk precursor or vitellogenin from the blood (Wiegand, 1982). Vitellogenin (VTG) is a very high density phospholipoglycoprotein complex synthesized, under oestrogen stimulation, by the liver of oviparous vertebrates where it probably binds calcium before being secreted into the bloodstream. In trout, vitellogenin contains 0.5% calcium (Campbell & Idler, 1980; Sumpter, 1985). It is sequestered from the blood by growing oocytes during the period of gonadal development (Tyler *et al.*, 1987, 1990) a process termed exogenous vitellogenesis. Vitellogenin has been isolated from the blood of a variety of fish (reviewed by Ng & Idler, 1983; Ho, 1991). The estimate of plasma vitellogenin molecular weight by electrophoresis varies between species from 380,000 in goldfish (de Vlaming *et al.*, 1977), to 420,000 in the medaka (Hamazaki *et al.*, 1987), to 470,000 in rainbow trout (Campbell & Idler, 1980), to 640,000 in stickleback (Ollevier & Covens, 1983). Vitellogenin has been identified and quantified directly and indirectly (Chapter 2). The present study followed changes in plasma calcium levels as an indicator of vitellogenic activity.

In salmonids, plasma/serum calcium concentrations and plasma/serum proteins have been shown to fluctuate with vitellogenic activity which is under the control of ovarian oestrogens (Elliott *et al.*, 1984; van Bohemen *et al.*, 1981). Oestradiol-17β (E–2) has been recognized as the most potent inducer of vitellogenin in fish (Sundararaj & Nath, 1981; van Bohemen & Lambert, 1981). It is secreted by the granulosa (follicle cells) and targets the liver where the hormone induces synthesis and secretion of vitellogenin which is sequestered by the oocyte and proteolysed into two yolk proteins: lipovitellin and phosvitin (Wallace, 1985). E-2 is also involved in the regulation of carbohydrate, lipid, and calcium metabolism (Fostier *et al.*, 1983). While E-2 stimulates production of vitellogenin by the liver, the hormone does not affect sequestration by the oocytes (Campbell & Idler, 1976). Vitellogenesis in fish is restricted to a distinct period of the reproductive cycle and normally occurs during the pre-spawning season (van Bohemen & Lambert, 1981; Lamba *et al.*, 1983; Scott & Sumpter, 1983a; Copeland *et al.*, 1986). The onset of vitellogenesis is often preceded by, or coincides with, a rise of oestrogen titre in the bloodstream, thereby providing evidence that the process is under oestrogenic control (Emmersen & Petersen, 1976; Elliot *et al.*, 1979; Idler & Campbell,

1980; van Bohemen & Lambert, 1981; Sundararaj *et al.*, 1982). The induction of vitellogenin synthesis (vitellogenesis) by the liver subsequent to E-2 injection has been reported for several species of fish (Plack *et al.*, 1971; Emmersen & Petersen, 1976; Terkatin-Shimony & Yaron, 1978; Elliot *et al.*, 1979; Nath & Sundararaj, 1981, Bradley & Grizzle, 1989, Burzawa-Gerard & Dumas-Vidal, 1991). A single injection of E-2 is sufficient to produce a long-lasting, dose-dependent rise in plasma vitellogenin level as demonstrated in the flounder (Korsgaard *et al.*, 1983) and the rainbow trout (van Bohemen *et al.*, 1982a; Bjornsson & Haux, 1985). Injection of E-2 has also been used in many fish to determine its effect on blood calcium and/or protein and/or vitellogenin synthesis and E-2 levels: in rainbow trout (Nagler *et al.*, 1987; Flett & Leatherland, 1989), Atlantic salmon (Olin & Von Der Decken, 1989a), spotted seatrout (Copeland & Thomas, 1988) and eel (Petersen & Korsgaard, 1989). E-2 injections could also help identify the fraction related to vitellogenin on an electrophoretic profile. Electrophoresis of serum/plasma on cellulose acetate yields five protein components, albumin, α_1-α_2-β and γ-globulins (Sargent, 1969). In human serum (Tietz, 1976), these fractions have been associated with the presence of specific proteins. The fastest migration, the albumin-type fraction, contains albumin. It is quantitatively non-predominant in Teleostei (Babin & Vernier, 1989) compared to the other fractions. The α_1 globulin fraction can include $\alpha_1$anti-trypsin, $\alpha_1$lipoprotein (HDL) and urosomucoid. While vitellogenin is a VHDL (Babin & Vernier, 1989), its band could be between the albumin and the α_1 fraction. The α_2-globulin fraction contains α_2-macroglobulin, haptoglobulin and pre-β-lipoprotein (VLDL). The β-globulin zone represents transferrin, haemopexin and β-lipoprotein (LDL) while the γ-globulin are immunoglobulins with antibody function. Three fractions, α_1, α_2 and β represent proteins involved in lipid transport while α_1 and β are also involved in hormones transport. A globulin peaking between the α_1 and α_2 bands has been associated with the binding of thyroid hormones. In humans, an increase in the α_1 band can accompany oestrogen supplementation; an increase in β band can be characteristic of high levels of oestrogen (Tietz, 1976).

Plasma/serum factors have been shown to fluctuate with the degree of ovarian development, peaking prior to or with spawning, and normally staying at low levels during the post-spawning (ovarian quiescence) period. Internal factors (reviewed by Billard, 1989) regulate reproductive processes, and a subtle interaction with exogenous factors serves to induce ovarian recrudescence, maturation, ovulation and spawning. Environmental modulations have been reviewed by Crim (1982), Wootton (1982), Stacey (1984), Billard & Breton (1985), Lam & Munro (1987) and Bromage *et al.* (1992b).

Most sexually-mature fish exhibit an annual pattern of gonadal development and spawning regulated by environmental and hormonal factors. Temperature and/or photoperiod have been shown to influence gonadal maturation of different vertebrates (Cloudsley-Thompson, 1961; Follett, 1978; Elliott, 1981) and invertebrates (Waddy & Aiken, 1984, 1992; Robertson *et al.*, 1991). Temperature and/or photoperiod have been recognized as the most important cues in the timing of gametogenesis in temperate species (Lam, 1983). The photoperiodic control of reproduction has been reviewed by Bromage *et al.* (1990, 1992b). While daylength may serve both a directly stimulatory effect on reproduction and a synchronizing effect on the endogenous rhythmicity (reviewed by Baggerman, 1980), temperature plays a major role in regulating reproduction (de Vlaming, 1974, Peter & Hontela, 1978; Peter & Crim, 1979; Scott, 1979; Bye, 1984; Whittier & Crews, 1987).

For many teleosts, the timing of gonadal development may result from a complex integration of temperature and its cycle, an internally programmed annual timer, and daylength. Other environmental considerations may be involved, but what seems important is the cycling of different events recognized by the fish and their use to trigger maturation, ovulation and/or spawning. Constant temperature and/or photoperiod regimes have also been used to modulate ovarian development and spawning (reviewed by Bromage *et al.*, 1992a,b), to express the endogenous rhythm (reviewed by Bye, 1984) or to entrain the endogenous rhythm (Davies *et al.*, 1991; Duston & Bromage, 1991). The environmental influences of temperature and/or

photoperiod on fish reproductive cycles have been investigated mainly for salmonids using constant and/or fluctuating regimes (Henderson, 1963; de Vlaming, 1972a,b; Breton & Billard, 1977; MacQuarrie *et al.*, 1978, 1979; Whitehead *et al.*, 1978b; Lundqvist, 1980; Bromage & Duston, 1986; Johnston *et al.*, 1987; Davies & Bromage, 1991). Similar studies on species with aquacultural potential, have increased in recent years (e. g. Leong, 1977; Lee & Hirano, 1985; Davies *et al.*, 1986; Richter *et al.*, 1987; Poncin *et al.*, 1987; Carrillo *et al.*, 1987, 1989; Cantin, 1987a,b; Lee *et al.*, 1987; Devauchelle *et al.*, 1987; McCarty, 1987; Devauchelle & Coves, 1988; Poncin, 1988, 1989; Walsh & Johnstone, 1992).

The primary goal of this research was to define the ovarian development and spawning of smallmouth bass in relation to constant, extended seasonal and natural temperature-photoperiod regimes. The secondary goal was to assess the apparent reproductive plasticity or adaptability of smallmouth bass as expressed through environmentally-specific reproductive strategies. The techniques employed in this research included histological sectioning, monitoring of morphological and physiological (plasmatic components) changes over at least two yearly cycles and monitoring of natural and controlled spawnings as essential steps in defining the ovarian development and the possible decisions faced by smallmouth bass. Clearly, a better knowledge of individual processes of the female reproductive cycle is of prime importance in management of populations and the ability to improve aquacultural techniques particularly those related to the control of reproduction.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Introduction

The experiments were conducted in the laboratories and commercial hatchery of Aquaresearch Ltd's located at North Hatley, Qc, (45°14'N, 71°58'W) Canada. Water recirculation systems were used to simulate summer and winter temperature conditions all year round. Founded in 1984, Aquaresearch Ltd was and is still the first and only commercial facility producing smallmouth bass for restocking, in closed systems, in Canada.

2.2 Systems: rearing and spawning

Water recirculation and environmental control systems maintained specific temperature-photoperiod conditions for nine groups of smallmouth bass. These fish were compared to a control group (tenth group) held in a pond under natural climatic conditions. Two different environmental simulation systems were installed in an indoor facility: a rearing module (Fig. 2.1) and a spawning module (Fig. 2.2). In two of the rearing modules, smallmouth bass were maintained for up to two years, under constant temperature-photoperiod conditions (Fig. 2.3 & 2.4). The regime of the third module (Fig. 2.5) simulated the natural conditions in Quebec (Fig. 2.6) but with a compressed winter. Each of the rearing modules contained, in parallel, two rectangular tanks with rounded ends, 2.26 m long by 0.85 m wide and 0.5 m high (1,110 L). The effluent end of each tank contained a sedimentation zone. Water flowed by gravity from the rearing tanks to a common mixing chamber (133 L) where make-up water was added by an automatic float assembly. A filter of geotextile material was suspended in the mixing chamber to collect suspended solids.

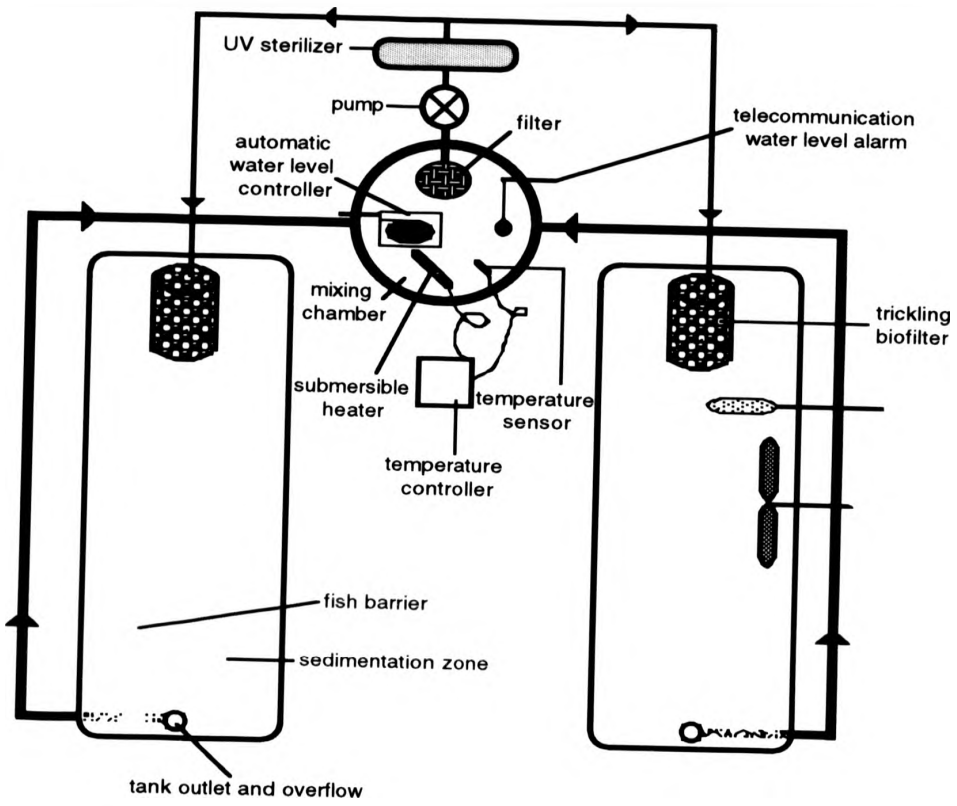


Fig. 2.1 Diagram (not to scale) of water recirculating rearing module used to simulate constant and cycling photoperiod-temperature regimes during two consecutive years. Water flowed by gravity to the mixing chamber and was pumped (25 L/min) back to the biofilters of each tank

Heaters and/or cooling elements were installed in the mixing chamber. Constant summer conditions (15L:9D; 22°C ± 1°C) were maintained with a temperature controller (model 74, YSI Thermistemp, Yellow Springs, Ohio), connected to five 200 W submerged glass heaters. Constant winter conditions (9L:15D; 10°C ± 1°C) were maintained by a temperature controller (model 63RC, YSI Thermistemp, Yellow Springs, Ohio), connected to a solenoid valve, which regulated flow of 3-4°C water to a heat exchanger in the mixing chamber. The heat exchanger consisted of three 17 m long coils (1.27 cm internal diameter) of #316 stainless steel. A 2 HP refrigeration system connected to an insulated stainless steel tank of 2,000 L maintained the water at 3-4 °C. Water was pumped to a 120 L constant pressure container from which it flowed through the cooling

systems and back to the central chiller when the solenoid valves opened. Flow rate through the solenoids was adjusted to minimize thermal excursions.

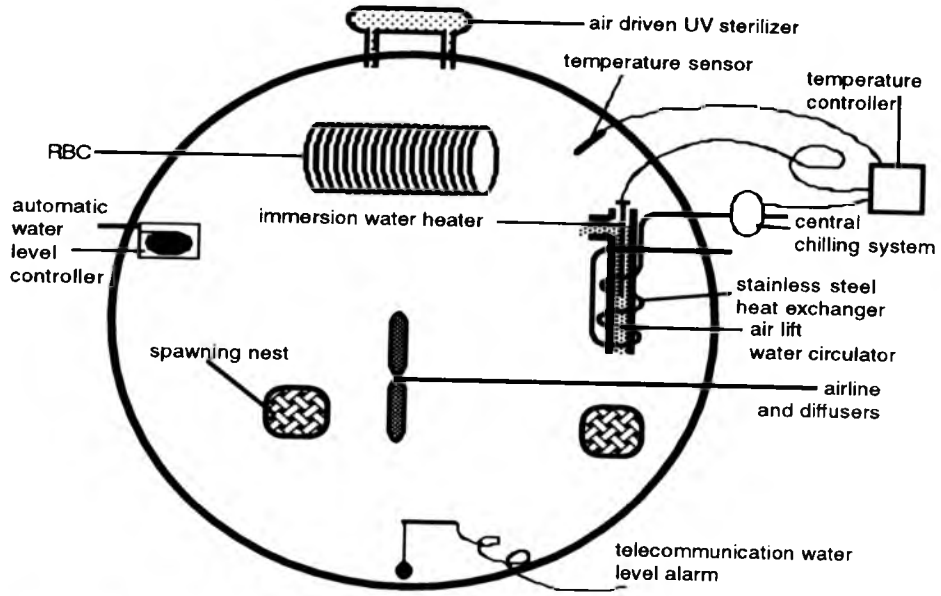


Fig. 2.2 Diagram (not to scale) of water recirculation system in the spawning pool used for controlled spawning. The RBC (rotating biocontactor) was moved by an air lift

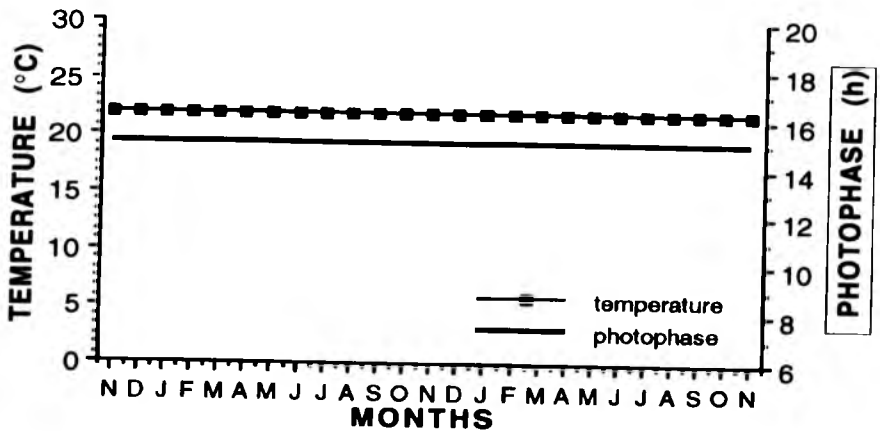


Fig. 2.3. Constant temperature and photoperiod regime used as summer conditions and related to the summer solstice in Quebec, Canada

The flow rate through each tank was 25 L min^{-1}. Sixty percent of the water was pumped from the mixing chamber through an ultraviolet sterilizer (model Q25IL, Aquanetics Systems, San Diego, California); 40% bypassed the sterilizer to protect beneficial (nitrifying and heterotrophic) bacteria from being killed. Water make-up was less than 6% of the system volume per day; this compensated for evaporation and the siphoning of uneaten food particles and/or accumulated feces.

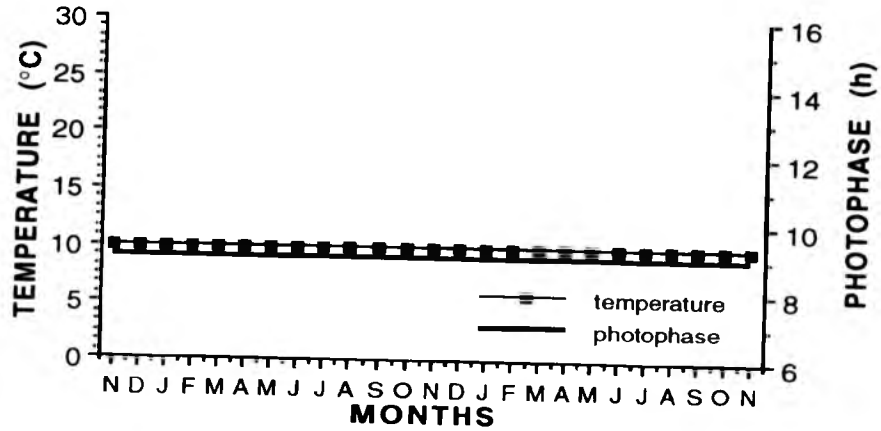


Fig. 2.4. Constant temperature and photoperiod regime used as winter conditions and related to the winter solstice in Quebec, Canada

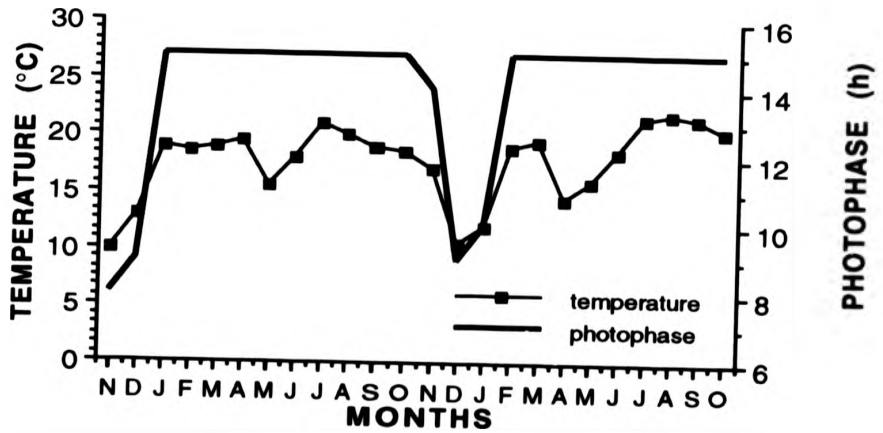


Fig. 2.5. Simulated seasonal temperature and photoperiod regime with a short winter period to induce early spawning

All of the water entered each tank through a packed column biofilter (36 cm diam. x 66 cm high, 0.07 m^3). The packing material consisted of polystyrene beads with a surface area of approximately 100 m^2/m^3. The role of a biofilter is principally to provide a substrate where nitrifying bacteria can grow and transform ammonia and nitrite into non-toxic nitrate. The major parameters that will affect biofiltration efficiency are: available filter surface and flow distribution, waste and hydraulic loading, cycling frequency and environmental variables such as temperature, pH, alkalinity and oxygen supply (Muir, 1982). All the water was filtered every 20 minutes. The biofilter was inoculated weekly with 2 ppm of a commercial mixture of nitrifying and heterotrophic bacteria, Bacta-Pur™ N-2000 (Aquaresearch Ltd, North Hatley, Quebec). The inoculations ensured the presence of an appropriate heterotrophic and lithotrophic community of bacteria, and ammonia (NH_3) and nitrite-nitrogen (NO_2-N) levels were maintained below recommended limits of 0.0125 mg/l and 0.06 mg/l (in hard water) respectively (Piper *et al.*, 1982).

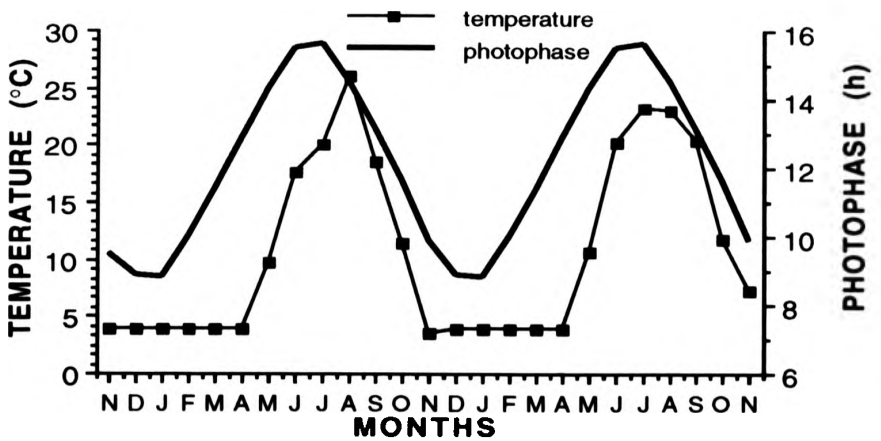


Fig. 2.6. Natural seasonal temperature and photoperiod regime in the pond found in Quebec, Canada (45° 14'N, 71°58'W)

Intermittent ozonation was used to breakdown "yellow substances" -long chain organic molecules that accumulate in low water exchange systems and which have low rates of bacterial biodegradation because of their large size (Rosenthal, 1980). Ozonation breaks the molecules, increases the rate of bacterial degradation and clarifies the water. Aeration

was provided by two air diffusers (15 cm x 3.8 cm x 3.8 cm) connected to a central air blower. Valves were used to control air flow and maintain oxygen levels of at least 6 mg/l.

The daylight intensity, at the water surface, was maintained at approximately 44 lux with a 60 W bulb installed over each tank and controlled by a rheostat. A Paragon home timer (McGraw Edison Ltd, Toronto, Ontario), regulated daylength, based on standard time. Each light-controlled room was isolated by black plastic curtains. As high light intensity can stress fish, studies that mention illumination levels, keep it generally at the minimum to maintain good feeding response (Ehrlich *et al.*, 1979). In this experimentation, low daylight intensity was maintained in both summer and winter conditions.

Smallmouth bass are naturally associated with structures in water and are often found in narrow spaces. Groups of ABS pipes (10 cm diameter x 45 cm long) were weighted and placed on the bottom of each tank to provide shelter and diminish natural aggression between the fish. Tanks were covered with a net to stop fish from jumping out.

Three fail safe security systems were employed to assure fish well-being. Firstly, an automatic generator maintained aeration and water supply through the make-up system during numerous power failures. Secondly, a water level alarm consisting of a float attached to a micro-switch was connected to a sound alarm and to a device which would call pre-registered phone numbers. Thirdly, each tank was provided with oxygen diffusers, connected to a tank of pure oxygen (medical grade), that could be manually opened to maintain oxygen levels during power failures if security system one failed to operate adequately.

The spawning module consisted of a circular tank, 3.6 m in diameter, containing 11,000 L of water furnished with a modified version of Inslee's artificial nests (Inslee, 1975) (Fig. 2.7).

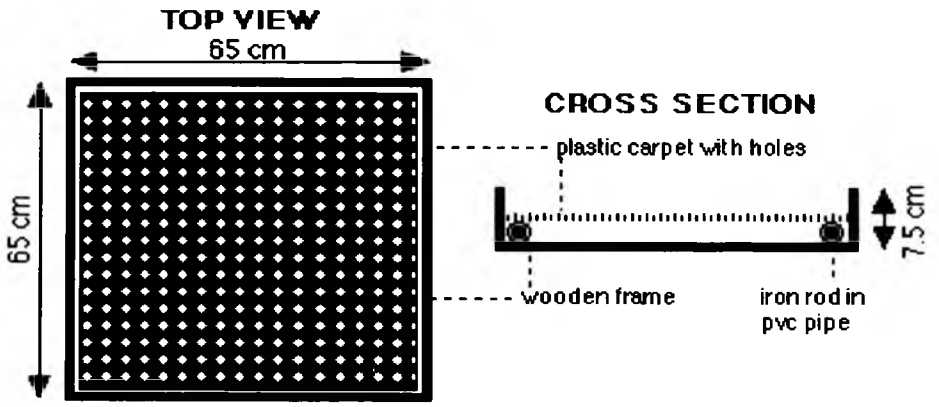


Fig. 2.7. Spawning nest made of screen covered with Astroturf type carpet used in controlled spawning

Rocks placed on a screen were replaced by a perforated carpet, Astroturf for fish culture (Monsanto, Ontario). An air lift pump circulated water through an ultraviolet sterilizer (model Q25IL, Aquanetics Systems, San Diego, California) back to the tank. A rotating biological contactor type filter (RBC) with 30 m^2 of surface, within the tank, was rotated by air injection. The RBC was inoculated the same way as the biofilters in the rearing systems. A temperature probe attached to the side of the tank was connected to a Set-Point Temperature Controller (model SP-LO, Heliotrope General, Spring Valley, California). This controlled either a solenoid valve, through which cold water flowed to a stainless steel heat exchanger which was 50 m long or a relay regulating a 2 kW immersion heater. The chilling system used the same central refrigeration system described above. Daylight was provided by 8 incandescent bulbs plus natural light. Light intensity varied between 40 and 350 lux depending upon sunlight. This increase in light intensity for the duration of spawning simulated natural conditions prevailing at spawning.

2.3 Rearing techniques: System maintenance and fish husbandry

2.3.1 System maintenance

The systems were visually checked daily. Excess food and feces were siphoned off daily and then the geotextile filter was washed. Shelters were cleaned every time smallmouth bass were sampled.

2.3.2 Water quality monitoring

Water recirculation systems, where more than 94% of the water was reused, required careful monitoring to insure good water quality. Temperature and dissolved oxygen were monitored daily with an oxygen meter (YSI, model 51B, Yellow Springs, Ohio). Total ammonia-nitrogen, nitrite-nitrogen and alkalinity were measured with LaMotte kits, models NANR, 0.02-1.0 mg/l, LNI, 0.02-0.3 mg/l and WAT-DR, 0-200 mg/l, respectively. These measurements were taken daily during the first three months of experimentation then reduced to two to three times a week. pH levels were recorded daily with a Piccolo pH meter (Hanna Quebec, Laval, Quebec). Ortho-phosphate, nitrate and conductivity were measured quarterly, with LaMotte test kits models VM-12, 1-10 mg/l, ENA (AG-32), 1-16 mg/l and YSI 33 S-C-T meter (Yellow Springs, Ohio), 0-500 µmhos/cm to insure that accumulation would not affect fish health. Limiting levels used were those established for salmonids (Piper *et al.*, 1982).

2.3.3 Fish Husbandry

Smallmouth bass used in this study were from a northern population (45°N), first and second generation in captivity. The first year, eighty-two mature smallmouth bass, three to eight years old, 28-42 cm in total length, were provided by Aquaresearch Ltd from its commercial broodstock and distributed on a temperature-photoperiodic history basis between all groups (groups A1, B1, C1, D1, N1) (Table 2.1). Both sexes were mixed together. The second year, one hundred and twenty two smallmouth bass, one to nine year old, 21-45 cm in total length included fish from the first year and first time spawners. They were divided into groups A2, B2, C2, D2 and N2 (Table 2.2). The eight groups (A1, A2, B1, B2, C1, C2, D1, D2) had different spawning and temperature-photoperiodic history (Table 2.3). It was not possible, during the first year, to follow all changes on an individual basis, because the external Carlin type tags used were constantly removed or damaged by the natural fish behaviour of scratching their back along the bottom or side of the tank. Loss of external tags in 80% of marked smallmouth bass was reported by Fraser (1955), in a field growth study. The present observations confirmed the limitations of using external tags in a long term study on smallmouth bass. During the second year experiment, each fish was individually tagged

with an internal passive tag (Passive Integrated Transponders, Biosonics, Seattle) inserted using a syringe sterilized in alcohol between each fish. This technique, which is used to follow salmon and steelhead smolt migrations and to identify different strains of largemouth bass broodstock (B. Ransom, pers. comm.), proved to be very effective for monitoring individual smallmouth bass. Ovarian development, during the same periods, was also followed in the ninth group (W), consisting of six fish in their third and fourth year of winter spawning (Cantin & Bromage, 1991). The last group, the controls (N1 and N2), consisted of a dozen smallmouth bass held in a pond where natural spawning occurred every year. Smallmouth bass, in the recirculation system as well as in the pond during the summer months, were observed at least once a day, when fed and/or when the tanks were cleaned.

Table 2.1: Description of photoperiodic-temperature and spawning history of smallmouth bass previous to the first year experiment

Groups	Temperature	Photoperiod	Spawning
A1	seasonal at 45° N, 71° W	seasonal at 45° N, 71° W	spring spawning
B1	seasonal at 45° N, 71° W	seasonal at 45° N, 71° W	no spawning observed
C1	seasonal at 45° N, 71° W	seasonal at 45° N, 71° W	no spawning observed
D1	seasonal at 45° N, 71° W	seasonal at 45° N, 71° W	spring spawning
N1	seasonal (Fig. 2.6)	seasonal (Fig. 2.6)	spring spawning
W1	two-month winter (Fig. 2.5)	two-month winter (Fig. 2.5)	winter spawning

Table 2.2: Description of photoperiodic-temperature and spawning history of smallmouth bass previous to the second year experiment

Groups	Temperature	Photoperiod	Spawning
A2	constant (22°C ±1°C)	constant (15L:9D)	spring spawning
B2	constant (22°C± 2°C)	constant (14L:10D)	first time spawners
C2	constant (22°C ±2°C)	constant (14L:10D)	first time spawners
D2	constant (10°C ±1°C)	constant (9L:15D)	spring spawning
N2	seasonal (Fig. 2.6)	seasonal (Fig. 2.6)	spring spawning
W2	two-month winter (Fig. 2.5)	two-month winter (Fig. 2.5)	winter spawning

2.3.4 Feeding

Bass were fed manually *ad libitum* six days a week with soft moist pellets from Rangen (Buhl, Idaho), Zeigler (Gardners, Pennsylvania), and/or Biodiet (Warrenton, Oregon) and/or a homemade mixture (Aquaresearch Ltd, North Hatley, Quebec) over the two year period. During the second year, a weekly meal of live goldfish completed their diet. The quantity of food consumed was recorded to assess fish health with regards to water quality. On any given day, fish from different conditions received the same diet although quantities could differ. Experimental studies have linked starvation and/or limited food ration to non-reproductive seasons in the winter flounder (Burton & Idler, 1987) and to decreased fecundity in rainbow trout (Scott, 1962). For these reasons, a food ration of 0.5 to 2% body weight was offered every day.

Table 2.3: Description of conditions and sampling of smallmouth bass in terms of number of fish per group, their temperature and photoperiod regimes, number of fish measured at each sampling date, number of fish and frequency of sampling for catheterization and histology, number of fish injected with oestradiol-17β, number of fish tested for spawning under each temperature-photoperiod regime and number of fish sampled for plasma analyses

SAMPLING									
Group	# Fish	•C	Photo-period	Lt & Wt # fish	Ov. Dev. Cath.	Ov. Dev. Histo	E-2 induc.	Spawn Test # fish	Plasma analyses
A1	16	22 °C const	15L:9D	16	3-8ind./4-6 weeks	1ind./4-6 weeks		6	5-7ind./4-6 weeks
B1	16	22 °C const	15L:9D	16	3-8ind./4-6 weeks	1ind./4-6 weeks			5-7ind./4-6 weeks
C1	16	10 °C const	9L:15D	16	3-8ind./4-6 weeks	1ind./4-6 weeks		6	5-7ind./4-6 weeks
D1	16	10 °C const	9L:15D	16	3-8ind./4-6 weeks	1ind./4-6 weeks		10	5-7ind./4-6 weeks
W1	6	comp wntr	seasonal with compr. winter	6	4ind./4-6 weeks			6	
N1	12	cyc.	cyclic	12	4ind./4-6 weeks	1ind./4-6 weeks		12	
A2	21	id. A1	15L:9D	21	3-8ind./4-6 weeks	1ind./4-6 weeks			
B2	33	id. B1	15L:9D	33	0-12ind./4-6 weeks	1ind./4-6 weeks	6		5-7ind./4-6 weeks

Table 2.3 Description of conditions and sampling of smallmouth bass (cont.)

C2	33	id. C1	9L:15D	33	3-8ind./4-6 weeks	1ind./4-6 weeks	6		5-7ind./4-6 weeks
D2	23	id. D1	9L:15D	23	3-8ind./4-6 weeks	1ind./4-6 weeks			
W2	6	id.W1	compr. cyclic	6	4ind./4-6 weeks			6	
N2	12	id. N1	cyclic	12	4ind./4-6 weeks	1ind./4-6 weeks		12	4-6 ind./4-6 weeks

2.4 Experimental design

Smallmouth bass were maintained for up to two years under natural (Fig. 2.6) and artificial (Fig. 2.3, 2.4 & 2.5) temperature-photoperiod conditions, to study their ovarian development and spawning strategy in relation to constant and seasonal temperature-photoperiod conditions as well as the influence of their previous spawning and temperature-photoperiod history. Sampling procedures (Table 2.3) on anesthetized fish included morphometric measurements, insertion of a catheter to collect oocytes and blood sampling from the Cuverian sinus (Section 2.5). In addition, the ovaries of fish sacrificed for the histology were weighed; the left ovary was preserved in Gilson's fluid (oocyte count) (Section 2.5.4) and the right ovary was preserved in Bouin (histology) (Section 2.5.3). Oestradiol-17β (E-2) was injected in selected fish to help identify the vitellogenin peak on the electrophoretic profile (Section 2.5.7).

The constant conditions simulated summer (15h:9h) and winter (9h:15h) solstices in terms of photoperiod with a differential in temperature of, at least, 10°C. At a latitude of 45° N, these temperature-photoperiod conditions are encountered by smallmouth bass each year for a short period of time. 10°C was maintained as the winter temperature condition, because personal observations and previous work by Webster (1954) and Bennett & Childers (1957) indicated that feeding activity of smallmouth bass was reduced below 10°C, and Scott (1962) and Bromage & Jones (1991) showed that

reduced feeding can affect fecundity and maturation respectively. Field observations of lakes from Ontario (Turner & MacCrimmon, 1970) and Wisconsin (Hile & Juday, 1941) have suggested that smallmouth bass prefer summer temperatures near 21°C. For these reasons plus the fact that the cooling system would be adequate to maintain this delta T° during the hot and humid summer months, the summer and winter temperatures were set at 22±1°C and 10±1°C, respectively. Seasonal conditions, for the winter spawning group, simulated a short winter (two to three months) and fluctuating temperature-photoperiod during a longer spring followed by natural summer conditions. Individuals from each group were monitored, during two consecutive years, for oocyte development and gonadal growth (condition factor (K)). Plasma changes in calcium, proteins and oestradiol concentrations were monitored during the second year. These parameters were used in an attempt to define the reproductive cycle of smallmouth bass and to assess the importance of temperature-photoperiod conditions as a "zeitgeber" for oocyte synchronization.

Individuals from groups A1, D1, W1, W2, N1 and N2 (Table 2.3) were also followed for their oocyte development as well as to estimate their fecundity, the number of eggs released per spawning and their spawning strategy. Fecundity, the number of ripening eggs in the female prior to the next spawning period (Bagenal & Braum, 1978) has been determined either by a count of total eggs removed at stripping *e.g.* in rainbow trout (Bromage *et al.*, 1992a), a count of eggs released at spawning *e.g.* sea bass (Prat *et al.*, 1990) or a direct count of ripe ova from the ovary *e.g.* speckled trout (Vladykov, 1956). A count of eggs released at spawning has been used because stripping the eggs has not yet been a successful technique in smallmouth bass. With ovarian autopsies, potential fecundity, adapted from Macer's work (1974), has been used when vitellogenic, mature and atretic oocytes were counted together. A common criterion used with serial spawning fish has been the presence or absence of yolk, a method used by Rao (1971) for Indian mackerel, Macer (1974) for the horse mackerel and more recently by Hunter *et al.* (1992) in Dover sole. Normally, atretic oocytes are subtracted, if identified. However, because the fish were prevented from spawning and atresia became quantitatively more important than under natural conditions, atretic oocytes were

included. Total count and relative count (total count/kg) of oocytes were also determined.

Finally, six selected females from groups B2 and C2 were injected with oestradiol-17β (E-2) to stimulate vitellogenin synthesis and to identify its peak on the electrophoretic profile; calcium levels were also monitored to evaluate its use as an index for vitellogenin secretion as observed in rainbow trout (Elliott *et al.*, 1984). Overall, the present study with constant conditions, aimed at defining the reproductive rhythms of smallmouth bass and delineating the role of temperature and photoperiod as cues and/or triggers of a particular phase of reproductive development.

2.5 Sampling and fixation techniques

2.5.1 *In vivo* measurements and oocyte sampling

Smallmouth bass were sampled every 4 to 8 weeks. Prior to manipulation, fish were starved for 24-32 hours. Manipulations occurred between 9:00 and 17:00 hours. Fish were transferred to a 100 L tank filled with water from their rearing module (same temperature) and transferred, two to five at a time, to a methyltricainesulfonate (MS-222) bath at a concentration ranging from 0.06 g/l to 0.09 g/l (60-90 ppm) depending of their size. During manipulation, which lasted about 1 1/2 to 2 hours per tank, light intensity was increased to a maximum of 140 lux to facilitate data collection. Total and standard length were recorded, to 1 mm, using a measuring board; fish less than 1 kg were weighed with an Ohaus balance, with a precision of ±2 g. A Terraillon balance with a 5 kg capacity and a precision ±5 g was used for larger fish.

Oocytes were collected using the catheterization technique (Ross, 1984). The catheterization consisted of gently inserting a plastic tubing (1.0 or 1.3 or 1.5 mm I.D.) into the urogenital opening through one oviduct. Gentle suction was applied long enough to collect the oocytes which were immediately fixed in 5% formalin. Minimum and maximum longitudinal sizes were later determined under a stereo microscope with a calibrated ocular micrometer. The sizes were then grouped into six stages, described in Section (3.2):

1. oogonia, <0.05 mm
2. previtellogenic stage
    2.a early and late perinucleolar, 0.05-0.4 mm
3. secondary growth
    3.a cortical alveolar, 0.40-0.70 mm
4. vitellogenic stage
    4.a early vitellogenic, 0.70-0.9 mm
    4.b vitellogenic, 0.9-1.7 mm
5. mature stage, 1.75-2.55 mm
6. atretic oocytes

All experimental fish were sampled all year round except for those in the pond (groups N1 and N2), which were sampled only from May to November. The November sampling of control fish was done on fish kept in a cage from early September. It is practically impossible to collect smallmouth bass in Quebec from the end of September to the end of April because of their lethargic behaviour below 10°C. The only technique that could work is electro-fishing which is, by regulation, exclusively used by the Department of Hunting and Fishing, Quebec Government. This technique is also limited if fish are resting between rocks which is often the case for smallmouth bass.

2.5.2 Blood sampling and plasma analyses

Smallmouth bass were sampled every 4 to 8 weeks. Prior to manipulation, fish were starved for at least 24 hours. Five to eight fish, from each condition, were transferred to a 100 L tank filled with water from their rearing module containing MS-222 at a concentration ranging from 0.06 g/l to 0.09 g/l (60-90 ppm) depending of their size. This sampling was carried out between 11:00 and 13:00h.

Blood was sampled from the Cuverian sinus using 1 ml tuberculin syringe with a gauge 23 needle. An anti-coagulant, heparin sodium (Organon Teknika, Toronto, Ontario) was drawn into the syringe and forced out several times to ensure that no heparin remained in the syringe except for a film on the walls. 0.5 to 1 ml of blood was collected from each individual and analyzed separately. To prevent haemolysis, blood samples were gently

expelled into a 0.5 or 1.0 ml centrifugation tube and centrifuged for two minutes in a Fisher Micro centrifuge, model 235B at 12,400 rpm. The plasma was transferred with a Pasteur pipette in a 0.5 ml or 1 ml centrifugation tube, capped, wrapped in Parafilm and frozen until analyzed. Preparation of the samples and all analyses were performed at the Laboratory of Clinical Biochemistry, Centre Hospitalier de l'Université de Sherbrooke, Sherbrooke, Qc.

Total calcium was used as an indicator of circulating vitellogenin and correlated with E-2 concentration. Vitellogenin has been identified and quantified directly by homologous radioimmunoassay (RIA) (Elliott *et al.*, 1984; So *et al.*, 1985; Sumpter, 1985), non-homologous radioimmunoassay (RIA) (Idler *et al.*, 1979; Campbell & Idler, 1980), gel electrophoresis (van Bohemen & Lambert, 1981; van Bohemen *et al.*, 1982a), immunoelectrophoresis (Goedmaker & Verbroom, 1974; Maitre *et al.*, 1985; Chen *et al.*, 1986) and immunodiffusion procedures (Plack *et al.*, 1971; Ueda *et al.*, 1984). Levels of vitellogenin have been estimated by the determination of serum total phosphoprotein phosphorus levels (Bailey, 1957; Whitehead *et al.*, 1978a; Elliott *et al.*, 1979; Craig & Harvey, 1984; Nagler *et al.*, 1987), of alkali-labile phosphoprotein phosphorus (Emmersen & Emmersen, 1976; Emmersen & Petersen, 1976; Hori *et al.*, 1979; Campbell & Idler, 1980; de Vlaming *et al.*, 1980; Tinsley, 1985; Nagler *et al.*, 1987), of total protein (Plack *et al.*, 1971; Emmersen & Emmersen, 1976; Yaron *et al.*, 1977, 1980; Tinsley, 1985) and of calcium (Bailey, 1957; Aida *et al.*, 1973; Yaron *et al.*, 1977, 1980; Whitehead *et al.*, 1978a; Elliott *et al.*, 1979; Hori *et al.*, 1979; Tinsley, 1985; Nagler *et al.*, 1987; Norberg *et al.*, 1989). Of all these studies, it is particularly Nagler *et al.* (1987) and Norberg *et al.*(1989) who showed that calcium increased proportionally with vitellogenin and sexual maturation for rainbow and brown trout, respectively.

Total calcium was measured with the Kodak Ektachem Clinical Chemistry Slides Technique (Eastman Kodak Co., Rochester, New York), using a dry, multilayered analytical element coated on a clear polyester support. The sample size was 10 µL of plasma. The analysis is based on the reaction of calcium with an indicator dye, Arsenazo III, in a buffer layer at pH 5.6. The amount of coloured complex formed is proportional

to the amount of calcium present in the sample and is measured by reflected light at 680 nm. The range of reading, on undiluted sample, is between 1.0-16.0 mg/dL, and Kodak Ektachem Calibrators were used to calibrate each series of assays.

Total protein was measured with Kodak Ektachem Clinical Chemistry Slides (Eastman Kodak Co., Rochester, New York), using a dry, multilayered analytical element coated on a clear polyester support. The sample size was 10 μL of plasma. The method of analysis is based on the biuret reaction, characterized by the generation of a violet-coloured complex when protein is treated with cupric ion in an alkaline medium. The reagent layer contains copper sulfate, tartaric acid and lithium hydroxide. The amount of coloured complex formed is proportional to the amount of total protein in the sample and is measured by reflected light at 540 nm. The range of reading is between 2.0-11.0 g/dL and Kodak Ektachem Calibrators were used to calibrate each series of assays.

Profiles of protein composition were performed on cellulose acetate gel-electrophoresis. Electrophoresis on cellulose acetate was developed largely through the studies of Kohn (1957). Advantages of cellulose acetate are sharper bands using small quantities of material (3 μL) and the speed of separation. However, because of the homogeneous gel material, it measures only the charge and mobility of the migrating proteins limiting the number of interpretable fractions (zones). This method is considered a semi-quantitative technique; the higher the ratio charge/mass of the protein, the faster it will migrate. Other electrophoretic techniques, like the more recent use of disc polyacrylamide gel where pore size can be tailored by changing the concentration of the gel, allow separation of the proteins based on their size, like a sieve, so molecular weights can be determined. It also separates the proteins into more fractions. But as described in Chapter 4, sensitivity of the cellulose acetate electrophoresis was sufficient to ascertain the presence of E-2 and/or a lipo-protein complex in the plasma. The cellulose acetate gel electrophoresis was performed on Sepraphore III membranes (Gelman, Montreal, Quebec) which were soaked in a buffer solution of Barbital (pH 8.6). Once the strips were blotted, they were placed on the support in the electrophoretic tank already filled with the buffer, and 3 μL of plasma sample was applied. The proteins were allowed to migrate for 20 minutes at

250 v. Colouration with Ponceau-S (Sigma Chemical Co., St-Louis, Missouri) followed with four successive washing baths in acetic acid (5%) and final clearing with Sepra-Clear II (Gelman, Montreal, Quebec). The membrane was then dried in an oven (80-90°C) for 20 minutes, hardened at room temperature and read at 520 λ for the densitometry. A control with human serum (MHA du Canada Inc., Pointe-Claire, Quebec) was run with each batch.

Oestradiol-17β (E–2) was determined with the no-extraction, solid phase ^{125}I radioimmunoassay (Coat-A-Count Oestradiol procedure) (Diagnostic Products Co., Los Angeles, California). The sample size was 100 µL. This technique is based on antibody-coated tubes where ^{125}I-labelled oestradiol competes with oestradiol in the sample for antibody sites. After an hour incubation at 37°C, the tube is counted in a gamma counter and the quantity of oestradiol is determined by comparing the counts to a standard curve. The calibration permits readings between 20-15,000 pg/ml. The assay can detect as low as 8 pg/ml with the three hour incubation procedure. The antiserum is highly specific for oestradiol with a relatively low cross-reactivity to other naturally occurring steroids. The highest percent cross-reactivity recorded was 1.1% for estrone.

An inter-assay standard, consisting of an unidentified mixed plasma sample was included with all second year samplings (Table 2.2). Levels of oestradiol-17β measured were all 10 pg/ml and less in this sample.

Table 2.4: Inter-assay control

Sample	Calcium (mg/dL)	Total protein (g/dL)
1	10.3	4.4
2	9.8	4.6
3	10.1	4.9
4	10.0	4.6
5	10.0	4.6
mean	10.0	4.6
standard deviation	0.18	0.18

Intra-assay calibration was done between each series of samples with the laboratory standard controls. The inter-assay control from the pooled plasma showed that the degree of variability between the data, expressed by the standard deviation, represented a variation of 1.8% for calcium determinations and 3.6% for total proteins concentrations. These differences in reproducibility were taken into account when blood parameters were discussed (Chapter 4).

2.5.3 Ovarian histology

Methods to assess ovarian development, reviewed by West (1990), classified histology as the most precise technique particularly when used in conjunction with oocyte size and gonad indices. One female smallmouth bass, from each constant condition, was sacrificed, every 4-8 weeks, in a MS-222 bath at 0.09 g/l, to collect the ovaries, which were weighed separately on a Mettler AE 260 Delta Range balance before fixing the right ovary in Bouin's solution (250 ml formalin 37%, 50 ml glacial acetic acid and 750 ml picric acid 1.2%) for twenty four hours. The ovary was then rinsed and stored in 70% alcohol until embedding.

The techniques used for histology were based on Humason (1979). The ovarian tissue fixed in Bouin was sectioned into three parts, distal, median and proximal. Each part was first dehydrated by immersing it in alcohol solutions of 90% and two baths of absolute alcohol for one hour per step followed by a last absolute alcohol bath with a pinch of anhydrous copper sulphate for two hours. Then clearing was achieved by immersing the ovary into three baths of xylene the two first lasting an hour each and the last one for two hours. The paraffin embedding with Paraplast-Plus followed with three sequential baths of a duration of a night, half-day, and half-day under vacuum. The temperature maintained was 48°C. The sectioned ovary was then transferred to an inclusion mold (Canlab, Montreal, Quebec) and embedded. The cubes were frozen prior to cutting. Sections of 4-8 µm were cut with a microtome Microm Heidelberg, model HM 320, with a disposable knife. In some cases, the block was covered with a solution of 1% cellodion before slicing. The sections were floated on a gelatin bath at 44°C, transferred on a slide and allowed to dry in a dry oven with formaldehyde. Three slides

of each distal, median and proximal sections were stained with Harris's haematoxylin and eosin (Bromage & Cumaranatunga, 1988) following the standard procedure (Humason, 1979). They were then mounted with an Eukitt solution and dried on a heating plate (45°C) prior to examination under a Nikon YS2 microscope. Microphotography was performed with a Nikon camera attached to the YS2 microscope.

The oocytes were classified into seven developmental stages and five atretic stages, described in Chapter 3:

1. oogonia,
2. previtellogenic stage
    - 2.a early perinucleolar,
    - 2.b late perinucleolar,
3. secondary growth
    - cortical alveolar or (yolk) vesicle/vesicular
4. vitellogenic stage
    - 4.a early vitellogenic,
    - 4.b vitellogenic,
5. post-vitellogenic (migrating nuclear stage)
6. atretic stages
    - 6.a pre-ovulatory oocytes
        - 6.a.1 alpha 1(α1) and alpha 2 (α2)
        - 6.a.2 beta (β)
        - 6.a.3 delta (δ)
        - 6.a.4 epsilon (ε)
    - 6.b post-ovulatory ovary
        - 6.b.1 gamma (γ)

2.5.4 Total count of oocytes (Gilson's fluid)

The left ovary of sacrificed fish was preserved in Gilson's fluid (17 ml nitric acid 70%, 4 ml glacial acetic acid, 20 g mercuric chloride, 70 ml alcohol 70% and 900 ml distilled water) for at least 6 months with occasional shaking to help separate the oocytes from the

ovarian envelope. The use of Gilson's fluid is a standard method to assess fecundity in trout and is complementary to histological sectioning. However, its use with a batch-spawner type fish where clutches of different sizes oocytes can develop into mature oocytes for a subsequent spawn in the same season is arguable. Fecundity is generally defined as the number of ripening oocytes and mature ova or eggs just prior to spawning. It represents the spawning potential; however in the case of a multiple or batch spawner, the delineation between resting and ripening oocytes adds a degree of difficulty in the estimation. Methods suitable for most temperate species do not necessarily work with multiple spawners (Bagenal & Braum, 1978). Three methods were tried: the first one was a direct count of all oocytes. Five ovaries were used to determine a reference number. The second method was an adaptation of Macer (1974) with the batch spawner *Trachurus trachurus* (L.) using a technique for sub-sampling plankton. Because of a larger range between the smallest and largest oocytes compared to Macer's work, sub-samplings were non-homogenous. Variations in means and standard deviation of sub-samples lead to a high number of samples necessary to obtain statistically significant reproducibility within a sample, almost equivalent to counting the whole sample. This technique was not used in subsequent investigation.

The third method used, this time for all the samples, was to sub-sample with a Folsom plankton splitter (plankton-divider) to reduce the effect of different sinking rate caused by a non-homogenous population of oocytes and obtain equal partitions. This technique, as pointed out by Thomas (1989), avoids the difficulties caused by the presence of different egg sizes occurring in an ovary. The relative percentage of each category of oocytes was correlated with the direct count and/or the number of oocytes collected by catheterization (Section 2.5.5).

The oocytes from the left ovary preserved in Gilson's fluid were rinsed in water then poured in the Folsom splitter as many times as necessary to obtain a total count of 200-400 oocytes. Between each separation, the splitter was carefully rinsed to make sure no oocytes remained. The eggs were measured to the nearest 0.05 mm under a stereo microscope, classified into four categories, previtellogenic, vitellogenic, mature and

atretic. Their number was recorded per category then multiplied by the number of separations. This technique was used to quantify the potential fecundity (number of vitellogenic, mature and atretic oocytes) and the total egg count.

2.5.5 Oocyte catheterization and its validation

The *in vivo* technique of collecting oocytes from the ovary has been used for different species including goldfish (Ross, 1984), milkfish (Juario *et al.*, 1984), sole (Ramos, 1986), grey mullet (Lee *et al.*, 1986), and smallmouth bass (Cantin, 1987b). However, validations of this technique as an *in vivo* assessment of ovarian maturity are limited to a few species: grey mullet (Shehadeh *et al.*, 1973) and Asian sea bass (Garcia, 1989). As part of this study, validation of *in vivo* assessment of ovarian maturity has been realized by comparing frequencies of stages of maturity and size observed from Gilson's preserved ovaries, histological sections and oocytes obtained by catheterization. The stages of maturity were pooled into three categories: previtellogenic-cortical alveolar, vitellogenic and mature because each method could not provide the same level of precision in the determination of the stages. The goodness of fit from stages frequencies and size data between the methods was compared by a Chi square test. Data from these two techniques were pooled together (Section 3.4) for graphs on stage frequencies and data from the catheterization and Gilson were pooled for the graphs on oocytes dimension.

2.5.6 Individual and group spawning

Spawning behaviour, nest productivity, potential and absolute fecundity, number of larvae collected and number of spawns per female were monitored. In fish that have group-synchronous ovarian development and produce multiple clutches, estimates of fecundity involve two distinct aspects: clutch size and clutch frequency (Conover, 1985; Heins & Rabito, 1986). The sequential sampling of individual females over few months has helped define these two aspects.

Smallmouth bass taken at different times of the year from the two constant regimes (Table 2.3) and cyclic conditions (Cantin, 1987b, c; Cantin & Bromage, 1991) were

transferred into the spawning tank and monitored for up to four months. Observations on temperature-photoperiod regime, time, frequency and duration of spawning and spawning behaviour were collected. Once spawning was observed, the eggs present in the nest were counted and a sample examined under a dissecting microscope for the presence of a fertilization membrane. After incubation and larval development, the pigmented larvae (commonly called blackbass) were counted and recorded as the nest productivity if more than one female was present in the spawning tank at the time of spawning. The progeny of one female was recorded as her absolute fecundity.

Natural spawning from smallmouth bass living in a pond served as a reference for nest productivity, timing, frequency and duration of spawning. The same population of spawners was followed during three consecutive years. Males had access to six (6) artificial nests (Table 2.7) and a sandy-rocky shore to make their nest. Nest formation, spawning activity and the presence of eggs were monitored at least twice a day from April to June. Blackbass (smallmouth bass post-larvae) were collected, enumerated and recorded as nest productivity.

2.5.7 Identification of vitellogenin peak on the electrophoretic profile

Oestradiol-17β concentrations were measured only during the second year and on a limited number of fish while protein profiles have been determined for all fish sampled. Ng & Idler (1983) demonstrated, in rainbow trout, that oestradiol-17β stimulates hepatic synthesis of vitellogenin, a lipoglycophosphoprotein with a molecular weight of 300,000 to 600,000. To identify the peak of this complex protein on the electrophoretic profile, six smallmouth bass maintained under summer conditions and six maintained under winter conditions received an injection of oestradiol-17β diluted in alcohol and arachis oil at a concentration of 500 µg/kg. A control group of four females from both conditions were injected with the vehicle alone. To determine a concentration of oestradiol-17β of 500 µg/kg that would modify the protein profile and permit the identification of the vitellogenin peak, a preliminary trial was performed with a few individuals. Plasma levels of calcium, an index of vitellogenin secretion in rainbow trout (Elliott *et al.*, 1984), the protein profile, total protein and oestradiol-17β were sampled at time zero, 7-d after

injection and 30-d later. Methods of fish husbandry, blood sampling and analyses followed those described in sections 2.3.3 and 2.5.2, respectively.

2.6 Data analyses

2.6.1 Condition factor (K), relative condition factor (RCF) and somatic condition factor (SCF)

Condition factor (K) is most commonly calculated as a function of the weight divided by the cube of the length $\{K=f(\frac{wt}{l^3})\}$. This equation assumes isometric growth of the fish, which means that the weight increases as the cube of the length or that the slope of length/weight (log/log) relationship is three. If growth is allometric then the slope of the length/weight relationship can be significantly different from three. Under these circumstances, the mathematical accuracy is maintained by using the calculated slope of the length/weight line as the power factor in the condition factor equation. LeCren (1951) pointed out the need for this correction when studying perch from Lake Windemere. He observed that fish from different localities, of different sexes and different maturity levels can require use of power factors different from three and used a relative condition factor. A relative condition factor (RCF) uses the slope of a computed regression line instead of three. Its use allows comparison of maturity levels between fish of different sizes by limiting the effect of length. Allometric growth under different environmental conditions can then be compensated. The RCF was used for fish kept under constant conditions.

A modified somatic condition factor (MKs) has been used to define the type of growth of smallmouth bass where Ws = W (total) - W(gonads) was calculated then related to the total length $\{MKs=f(\frac{ws}{l^3})\}$. It is based on the method of Iles (1984) where the fish are weighed without their internal organs (liver, intestine and gonads) to define partial condition factors and evaluate cycles of metabolic components in relations to seasonality of feeding and growth. In the study with smallmouth bass, only the weight of the gonads were recorded.

Weight and length of each group have been computed to calculate the slope of the weight-length relation. The analysis of covariance (Dixon & Massey, 1957) was used to determine the differences between each group and the Student's-t test (two-tailed test) (Snedecor & Cochran, 1967) to compare the pooled slopes with the value three for isometric growth.

2.6.2 Gonadosomatic index (GSI) determination

The gonadosomatic index, also known as the gonosomatic index (de Vlaming *et al.*, 1982) or maturity index (Morse, 1981) is commonly expressed as the GSI. It is the relation of ovarian weight to somatic weight and provides a measure of spawning readiness or an approximate measure of energy expended for reproduction (Wilk *et al.*, 1990). It is also a common way to document the development or maturity of the gonads, and to measure the seasonal change in gonad growth. The ovaries from the fish sacrificed for histology (section 2.3.3) were individually weighed on a Mettler AE 260 Delta Range balance to 0.01 g precision to calculate the gonadosomatic index as:

$$GSI = \frac{100(L.O. + R.O).}{W.}$$

where GSI: gonadosomatic index,

L.O.: left ovary (g),

R.O.: right ovary (g), and

W: weight (g).

2.6.3 Data analyses

The comparison between female smallmouth bass maintained under constant conditions, cycles (Cantin, 1987a,b, 1988; Cantin & Bromage,1991) and a natural environment was assessed in terms of seasonal relative condition factor changes, gonadosomatic index, oocyte and ovarian development and spawning, calcium determinations, total plasma protein, protein profile with respect to vitellogenin peak and oestradiol 17-β concentrations. Spawning strategy was determined with data from histology, egg counts and staging (potential fecundity and ovarian catheterization), eggs spawned (absolute

fecundity) and number of larvae collected (nest productivity) as well as the timing and the number of spawnings per female. Temperature-photoperiod conditions at spawning were also correlated to spawning strategy. The effects of temperature and photoperiod regimes were assessed in terms of ovarian development and plasma components. Data were compiled and analyzed on a Macintosh system using Microsoft Excel 3.0 and graphed with Cricket Graph, Delta Graph and Microsoft Excel. Text and tables were produced with Microsoft Word 5.1 (Macintosh). The dependent and independent variables were transformed to logarithms to facilitate interpretation, in cases where the experimental relationship between a variable and a response resembled an exponential form (Alderdice, 1972). When polynomial regression curves were plotted to describe a relationship between the time and a particular response, third order equations were used to define seasonal patterns which, under normal circumstances, would show two points of inflexions.

CHAPTER 3

SPAWNING FOLLOWING NATURAL AND CONTROLLED CONDITIONS

3.1 Introduction

Smallmouth bass are spring spawners. There are many field investigations on their spawning (review Coble, 1975) and the factors affecting it (review Pflieger, 1975). Male bass move into spawning areas when water temperatures approach 15°C, and females join them as soon as the nest is constructed, or later, depending on water temperature and/or their ripeness (Coble, 1975). Field studies including observations on fecundity (Reynolds, 1965; Clady, 1970), spawning (Langlois, 1935; Reynolds, 1965, Inslee, 1975), behaviour and reproductive success have been extensively covered for northern (Rawson, 1945; Webster, 1954; Forney, 1972; Hoff, 1991) as well as for southern populations (Surber, 1943; Cleary, 1956; Funk & Fleener, 1974; Reynolds & O'Bara, 1991). Coble (1975) reviewed these studies. However, while there has been an impressive number of field studies on different aspects of the reproduction of smallmouth bass, there are many contradictory or only partially explained results. This is particularly true with respect to the number of eggs spawned, the number of spawnings and the timing. The purpose of this section is to help define the pattern of female reproduction by comparing spawnings under natural and controlled conditions as well as individual spawnings.

3.2 Natural spawning

3.2.1 Potential fecundity, total egg count and nest productivity

Smallmouth bass potential fecundity, numbers of vitellogenic, mature and atretic oocytes counted after Gilson's preservation, and total egg counts showed a high variability between individuals. The data collected from some natural spawners showed variation from a minimum of 1,880 to a maximum of 49,032 eggs (Table 3.1). Total egg count and total egg count/kg indicated the equivalent of one potential batch of eggs still remaining in the female at the end of the natural spawning period (June) followed by an

increase in numbers of maturing oocytes toward the end of the summer season. They did not show any correlation with the weight of the female and were different from the average number (17,150 eggs/kg) reported by Scott & Crossman (1974).

Table 3.1 Fish weight, numbers of previtellogenic (including cortical alveoli stages), vitellogenic and mature and atretic oocytes (oo), potential fecundity, total egg count and total egg count per kilo of control spawners (group N2) kept in a pond and sampled during summer

Date	Fish weight (g)	# prev. oo.	# vit. & mat. oo.	# atr. oo.	Pot. fec.	Total egg count	Total egg count / kg
June	208	121	4,428		4,428	4,549	21,870
June	214	300	2,130		2,130	2,430	11,355
June	188	190	1,880		1,880	2,070	11,011
Aug	875	16,400	1,792	128	1,920	18,320	20,937
Aug	502	15,321		896	896	16,217	32,305
Aug	390	7,542		512	512	8,054	20,651
Sep	482	8,944	15,208		15,208	24,152	50,108
Sep	1285	6,600	10,112		10,112	16,712	13,005
Sep	1250	10,384	16,640	512	17,152	27,536	22,029
Sep	760	14,592	5,888		5,888	20,480	26,947
Nov	844	13,400	49,032		49,032	62,432	73,972*
					MEAN	**18,450**	**25,208**

* not included in the calculation for the mean value

Nest productivity was also highly variable. Nest productivity (number of blackbass collected per nest) varied from a few survivors to a few thousands (Table 3.2). Spawning temperature was within the range reported in the literature; peak of spawning occurred between 16-18°C. The preferred spawning temperature did not stabilize near 18°C during the second year as it did during the other years, and the recorded nest productivity was lower.

Table 3.2 Spawning temperature, spawning time and nest productivity (number of pigmented larvae collected per nest) in control pond over three years

YEAR 1: in control pond

Nest no	Temp. (°C)	Temp. May(°C)	Temp. June(°C)	Date of spawning	Date of collection	# of blackbass collected/nest
E-1	18			11 May	25 May	695
Beach	18			11 May	24 May	1,986
E-2	16			12 May	30 May	2,578
N	17.5			17 May	8 June	0
TOTAL						**5,229**
MEAN		18.9	20.7			**1,307**

YEAR 2: in control pond

Nest no	Temp. (°C)	Temp. (°C) Apr.	Temp. (°C) May	Temp. (°C) June	Spawning Date	Collection Date	# of blackbass collected/nest
N-O	21.5				22 Apr	25 May	585
O	14				9 May	1 June	0
E-1	17.5				15 May	30 May	1,248
Beach	22				8 June	20 June	471
N	19				9 June	21 June	1,446
N-E	22				13 June		0
E-2	23				18 June		0
TOTAL							**3,750**
MEAN		14.3	22.1	23.3			**536**

YEAR 3: in control pond

Nest no	Temp. (°C)	Temp. (°C) Apr.	Temp. (°C) May	Temp. (°C) June	Spawning Date	Collection Date	# of blackbass collected/nest
E-1	17				11 May	25 May	2,052
E-2	18				14 May	24 May	1,292

Table 3.2 Year 3: in control pond (cont.)

E-3	18				15 May	25 May	0
E-4	18				18 May	9 June	687
N-O	24				30 May	17 June	15*
N-E	19.5				8 June	19 June	1,278
O	21.5				29 June	5 July	6
TOTAL							**5,330**
MEAN		9.8	18	22.8			**761**

* nest was covered by fungus.

3.2.2 Timing of spawning and female reproductive tactics

During the three years that spawnings were followed in the control pond with the same group of fish (Fig. 3.1), spawning activity lasted for up to two months. There was only a short 10-day spawning period in the first year. However, spawnings were observed from the end of April to the end of June and were associated with temperatures between 14-24°C. Clusters of spawnings and intervals of 1, 2, 3 and 4 weeks between batch-spawning were not unusual. More than one batch of eggs on the same nest at a few days intervals was also common. Forty four percent of the spawnings occurred within 3-5 days of the full moon. New eggs were observed on nests in the morning and at the end of the afternoon indicating that spawnings were not limited to nocturnal activity.

3.3 Controlled spawning

3.3.1 Potential fecundity, absolute fecundity, total egg count and nest productivity

Potential fecundity from groups A1 to D2 (Table 3.3) indicated a larger number of ripe oocytes under winter conditions (groups C1, C2, D1, D2). The mean total egg count for the winter groups was quite similar to the one of summer groups (26,156 and 25,991 respectively), but they had a different distribution. Total egg count was higher in first time spawner kept in constant summer conditions while under constant winter conditions, the egg count was higher for the experienced spawners. The number of atretic oocytes was higher in second year constant winter conditions (D2) and first time spawners under summer conditions.

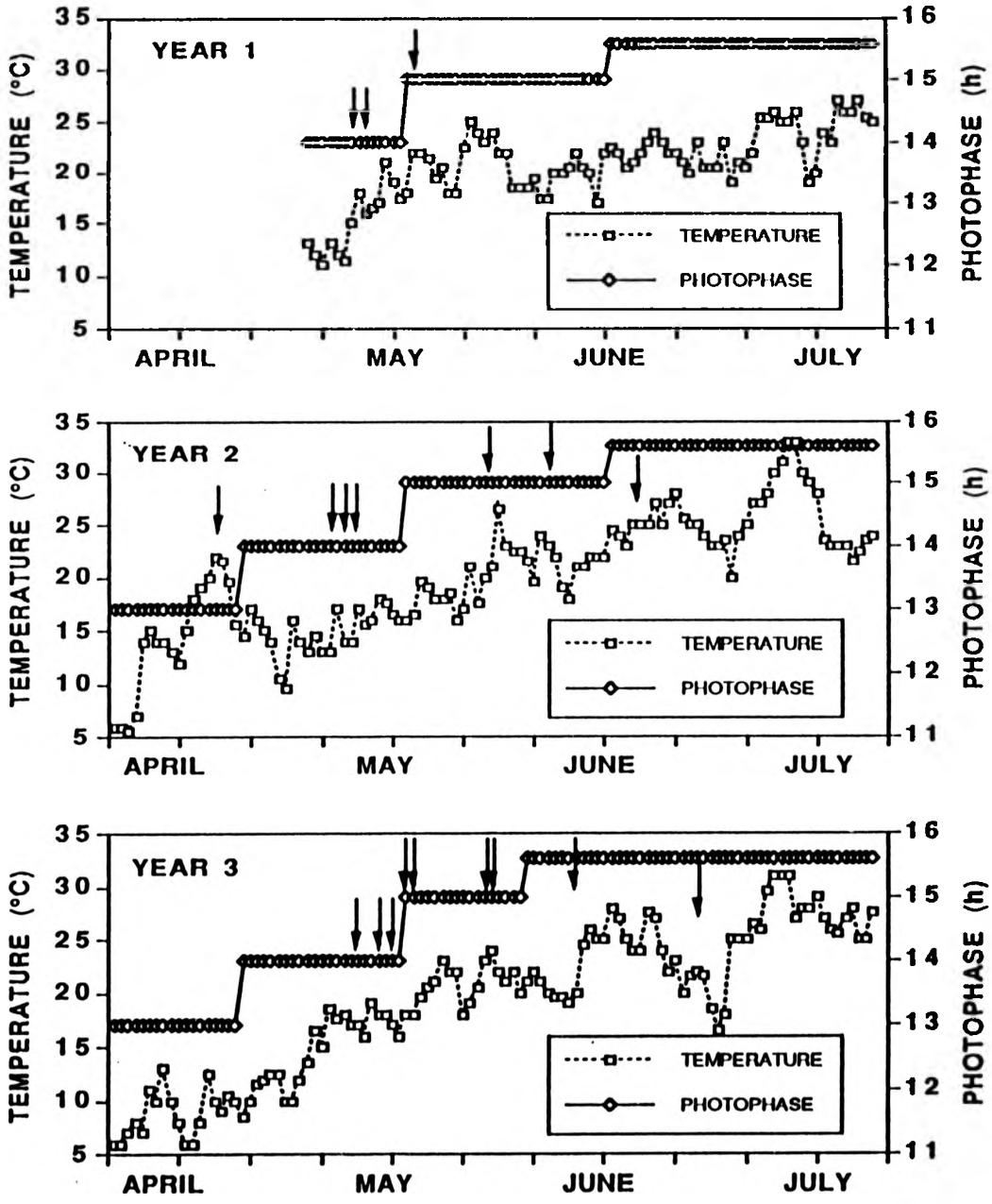


Fig. 3.1 Variations in temperature and photophase in control pond. The arrows indicate spawnings

Table 3.3 Potential fecundity and total count of oocytes in groups A1 (spawners under constant 22°C- 15L:9D photoperiod), A2 (spawners into their second year of constant 22°C- 15L:9D photoperiod), B1 (non-spawners under constant 22°C- 15L:9D photoperiod), B2 (first-time spawners under their second year of constant conditions), C1 (non-spawners under constant 10°C- 9L:15D photoperiod), C2 (first-time spawners into their second year of constant conditions) D1 (spawners under constant 10°C- 9L:15D photoperiod) & D2 (spawners into their second year of constant 10°C- 9L:15D photoperiod)

Condition	Date	Weight (g)	# prev. oo.	# vit. oo. & mat.	# atr. oo.	Pot. fec.	Total count	Total count / kg
A1	Nov. 88	810	18,672	13,312	5160	18,472	37,144	45,856
A1	Jan. 89	756	6400	8704		8704	15,104	19,978
A1	Mar. 89	1078	8848	19,168	5120	24,288	33,136	30,738
A2	Jan. 90	905	30,208	1408	1920	3328	33,536	37,056
A2	Apr. 90	572	10,700	2600	6500	9100	19,800	34,615
A2	Jul. 90	872	27,616	384		384	28,000	32,110
A2	Sep. 90	884	23,927	12,883		12,883	36,810	42,213
B1	Nov. 88	790	8768	10,496	3328	13,824	22,592	28,597
B1	Jan 89	1050	5904	37,760		37,760	43,664	41,584
B1	Mar. 89	612	7600	7168	6016	13,184	20,784	33,960
B2	Oct. 89	120	7800	2960		2960	10,760	89,666
B2	Jan. 90	426	12,400		17,600	17,600	30,000	70,422
B2	Apr. 90	212	6328		780	780	7108	33,528
B2	Jul. 90	598	10,000	8960	128	9088	19,088	31,919
B2	Sep. 90	374	17,816				17,816	47,636
C1	Nov. 88	764	9280	18048	2688	20,736	30,016	39,288
C1	Jan. 89	758	8544	9376	1792	11,168	19,712	26,005
C1	Mar. 89	548	6656	15969	1408	17,377	24,033	43,855
C2	Oct. 89	120	7800	2960		2960	10,760	89,666

Table 3.3 Potential fecundity and total count of oocytes (cont.)

C2	Feb. 90	316	2432	8544	2400	10,944	13,376	42,329
C2	Jul. 90	248	1024	5504		5504	6528	26,322
C2	Sep. 90	290	2600	8448	1408	9856	12,456	42,952
D1	Nov. 88	810	18,672	13,312	5160	18,472	37,144	45,856
D1	Jan. 89	784	20,160	14,336	3840	18,176	38,336	48,898
D1	Mar. 89	778	22,392	14,328	1536	15,864	38,256	49,172
D2	Apr. 90	604	9964		2848	2848	12,812	21,212
D2	Jul. 90	1012	10,752	34,048	6400	40,448	51,200	50,593
D2	Sep. 90	850	13,512	14,848	1664	16,512	30,024	35,322
AVG A1					**3427**	**17,154**	**28,461**	
AVG A2					**2105**	**6423**	**29,536**	
AVG B1					**3115**	**21,589**	**29,013**	
AVG B2					**3702**	**6086**	**16,954**	
AVG D1					**3512**	**17,504**	**37,912**	
AVG D2					**3637**	**19,936**	**31,345**	
AVG C1					**1962**	**16,427**	**24,587**	
AVG C2					**952**	**7316**	**10,780**	

Controlled spawning using photoperiod and temperature was attempted on different occasions but was successful only with smallmouth bass that had experienced winter conditions during the previous year. The fish showed individual differences as well as a multispawning capacity (Tables 3.4, 3.5 & 3.6). No spawning was observed for group C1 during the same periods. In group D1, spawning occurred independently of photoperiod and at water temperature ranging between 17-24°C.

The results, particularly during the second test, (Table 3.6) indicated clearly that a female can spawn more than once. They also showed that fecundity is highly variable between individuals and can be a limited tool in assessing blackbass survival. The number of eggs released and the weight of the female did not show any correlation.

Table 3.4 Nest productivity (number of post-larvae collected per nest) of Group W (winter spawners) with 2 male: 4 female spawners during three consecutive years

Year 1		Year 2		Year 3	
Date	Nest prod.	Date	Nest prod.	Date	Nest prod.
19 Jan	2,907	2 March	996	18 April	1,683
20 Jan	1,005	2 March	66	22 April	350
19 Feb	50	21 March	1,246	25 April	49
		21 March	366	27 April	2,425
				1 May	685
				8 May	2,001
TOTAL	**3,962**	**TOTAL**	**2,674**	**TOTAL**	**7,193**
AVG/NEST	**1,321**	**AVG/NEST**	**668**	**AVG/NEST**	**899 ***

* average is based on 8 nests whereas 2 nests had 100% egg mortality

Table 3.5 Absolute fecundity (total number of eggs released per spawning) and nest productivity (number of post-larvae collected per nest) of selected smallmouth bass (smb) from group D1 (9L:15D) under lond and short photophase, 2 male:4 female spawners

Group D1, long photophase (16L: 8D)			Group D1, short photophase (9L: 15D)		
Spawning Date	# eggs released	# fish collected	Spawning Date	# eggs released	# fish collected
4 June	1,826	1,467	16 May	3,900	185
11 June	1,522	1,219		3,291	1,575
18 June	2,001	1,800	17 June	2,700	0
20 June	1,006	575		1,225	567
			19 June	97	0
				326	133
TOTAL	**6,355**	**5,061**	**TOTAL**	**11,539**	**2,460**
AVG/NEST	**1,588**	**1,265**	**AVG/NEST**	**1,923**	**410**

Data from 11 spawnings of 4 females, in Aquaresearch Ltd's bass hatchery, showed a batch with a maximal number of 8385 eggs hatching into 6733 larvae (Fig. 3.7). Each female can spawn more than once and may be able to spawn over 10,000-15,000 eggs during the spawning season.

3.3.2 Timing of spawning and female reproductive tactics

Smallmouth bass spawned, in the hatchery, from January to the end of June independently of the photophase (Table 3.4, 3.5 & 3.7). Individual spawnings indicated that the same female can spawn more than once per season (Table 3.6) and at different times of the year if the right developmental and spawning conditions are met. Dates of controlled spawnings correlated with the phase of the moon indicated that 51% of the spawnings occurred 3-4 days preceding or following a full moon. However, when the spawnings from fish acclimated to constant conditions (groups A2 and D2) were compiled together, the percentage increased to 76%.

At the end of the experiments, the fish left from all the groups were stocked, in mid-July, into a lake. August and September spawnings were then observed. These spawnings occurred with temperature fluctuations between 15-18°C and decreasing photoperiod.

Table 3.6 Absolute fecundity (total number of eggs released per spawning) and nest productivity (number of post-larvae collected per nest) of individual females during spawning test. Female weights and groups were recorded. Group A1: regime with constant 22°C-15L:9D photoperiod; D1: regime with constant 10°C- 9L:15D photoperiod & D2: spawner into its second year of constant 10°C- 9L:15D photoperiod. The ratio male: female was 1:1

Duration of test	Origin	Spawning Date	Fem. W (g)	# eggs released	# fish collected
20 Sept 90 - 4 Feb 91	group D2	28 Dec	1,100	1,543	90
3 Oct 89 - 29 Nov 89	group D1	12 Oct	1,145	5,518	2,729
		15 Nov		3,520	2,233
29 Nov 89 - 31 Jan 90	group D1	7 Dec	1,015	1,233	0

Table 3.6 Absolute fecundity and nest productivity (cont.)

		30 Dec		454	24
8 Feb 89 - 10 March 89	group A1	15 Feb	660	3,125	2,125
12 March 89 - 30 Apr 89	group A1	13 Mar	940	797*	0**
TOTAL				**16,190**	**7,201**
AVG/NEST				**2,312**	**1,200**

* eggs not fertilized.

** nest ignored for average calculation

Table 3.7 Absolute fecundity (total number of eggs released per spawning) and nest productivity (number of post-larvae collected per nest) of spring smallmouth bass spawners from Aquaresearch's fish hatchery, 2 male:4 female spawners

Date of spawning	# eggs released	# fish collected
24 April	4,564	3,496
2 May	8,385	6,733
7 May	3,265	3,075
16 May	5,428	4,342
17 May	798	116
28 May	5,334	2,834
5 June	3,050	0
10 June	742	500
11 June	891	18
12 June	2,607	723
16 June	441	91
TOTAL	**35,505**	**21,928**
AVG/NEST	**3228**	**1993**

3.4 Discussion

Intraspecific variation in fecundity, potential or absolute, total egg count and nest productivity was observed under natural as well as controlled conditions. Evidence of differences in fecundity between different strains/populations have been reported in many species, particularly in wild stocks of brook trout (Vladykov, 1956; Gibson *et al.*, 1976), in 12 domesticated stocks of rainbow trout (Bromage *et al.*, 1990), in Atlantic salmon (Baum & Meister, 1971), in coho and chum salmon (Beacham, 1982), in salmonids in general (Rounsefell, 1957), in walleyes (Wolfert, 1969), in Atlantic cod (May, 1967), in the horse mackerel (Macer, 1974), in the sole (Devauchelle *et al.*, 1987) and in the Pacific herring (Nagasaki, 1958). Some of these studies have also shown that the number of eggs released or in development for the next spawning varied widely within a population and offers the potential for selection for husbandry or fishery goals.

The number of eggs released per spawning by smallmouth bass has been reported to vary between 5,000 and 14,000 per fish (Scott & Crossman, 1974). However, the data collected here from some natural spawners showed greater dispersion with a minimum of 1,880 to a maximum of 49,032 eggs (Table 3.1). While an average of 2,000 blackbass (Scott & Crossman, 1974) can be expected from one nest, numbers from 0 to 12,000 blackbass (Langlois, 1932) are not unusual.

Clady (1970) in his assessment of smallmouth bass fecundity counted between 2,000 and 9,500 clear, large "maturing" eggs. He also observed 892 to 3,637 eggs per nest hatching into 230 to 1,004 fry per nest. One of his explanations for the low deposition of eggs was that females would lay only part of their eggs and retained and resorbed the remainder. He raised the question about how a population of fish, which routinely spawns only a portion of its eggs could evolve through natural selection and did not further explain. What could have been missed here, is that having the capability of spawning as long as the conditions are favourable indicates flexibility in reproduction. Furthermore, it would permit exploitation of peaks in plankton cycles thus enhancing larval survival. This hypothesis, the "match-mismatch" hypothesis of Cushing (1967), is one of several put forward to explain the timing of fish reproduction. When compared

to a batch spawner like the mackerel which might be expected to produce between 20-70 batches per season, each batch with 7,000 to 19,500 eggs (Walsh & Johnstone, 1992), smallmouth bass might just be displaying reproductive plasticity.

It is also possible that not all the females will get to spawn. This fact has been observed in other species (eg. Kestemont *et al.*, 1991). Baylis *et al.* (1991) suggested that because all adult smallmouth bass do not have access to a nest, entire generations within a lake could be the product of a single pair. This may seem far fetched particularly when males commonly spawn with more than one female; however considering the intraspecific ovarian heterogeneity expressed by the number of mature eggs prior to spawning time, it appeared true that not all females would be ready for spawning at the same time. Asynchrony in female maturity is, again, one way to ensure that there are some individuals always ready to spawn when conditions become favourable. Milton & Blaber (1991) reported, in their studies of engraulid and dussumierid reproduction, that the spawning season of these fish is protracted while some individuals in the population can spawn at any time during the year. Spawning periodicity is also linked to exogenous factors (Chap. 6).

An indicator of spawning success, nest productivity, can be dependent on climatic or physico-chemical conditions as well as intraspecific spawning strategy. Temperature, wave action, fungus infection, capture of guardian male bass, predation, to mention a few, have all been cited as factors reducing recruitment (Fraser, 1955). Fungi have been reported as a major cause of mortality of smallmouth eggs in many studies (Clady, 1970; Coble, 1975; Inslee, 1975). An abrupt drop in temperature leading to the desertion of the nest by the male (Latta, 1963) has also been observed to cause egg mortality. Second nesting or multiple release of eggs over two months has been directly correlated with low temperature. When temperatures decrease suddenly below 15°C, egg mortality and second nesting have been observed. When temperatures are maintained so that they oscillate between 15° and 20°C, spawning is most often induced when temperature approaches 18°C, increasing or decreasing. Neves (1975) reported a second spawning period following a drop in temperature in Lake South Branch, Maine. Ridgway &

Shuter (1991) have demonstrated that nesting asynchrony is influenced by the rate at which temperatures increase in spring. In the present study, clusters of spawnings have been recorded when temperatures remain in the range of the 18°C for extended periods of time suggesting that temperature could help synchronize spawnings as it also provide a normal egg development. Kerr (1966) found that smallmouth bass eggs developed normally between temperatures of 15-25°C and that survival was reduced at lower and higher temperatures. The temperature increased gradually, in years 2 & 3, leading to a longer spawning season than in year 1.

Ridgway & Shuter (1991) suggested that the size of females influences the timing of spawning; larger females spawning first. It is also conceivable that the spawning pattern changes with the size of a female. In the few cases where size of females and spawning strategy could be correlated, females weighing at least a kilo spawned more than once but were not necessarily the first to spawn. However, the total oocyte count and the presence of different developmental stages within the ovary (Chapter 4) was similar in females of all sizes.

Observations of August and September spawnings and the spawning experiment under short photophase (9L:15D) clearly indicated that this photoperiod did not prevent spawning. Natural spawning occurs in spring (April to July) when the photophase is long and increasing. Under controlled conditions, induced spawning normally occurred when photophase was at least 14 hours. It appears that while specific temperature requirements are necessary to allow spawning, short or long photophase at the time of spawning do not affect spawning (Chapter 6).

Cantin (1987b) suggested that space requirements could limit spawning. Shuter & Ridgway (1991) showed that a lack of adequate substrate, of a quiet nesting area and/or territorial behaviour can also limit spawning. Cantin (1988) demonstrated that spawning is induced in ripe females by decreasing temperature to 15°C and then increasing to 20°C. Inslee (1975) mentioned that a water change in the spawning pond would stimulate spawning and reduce the incidence of fungus growth.

The factors presented here and in other studies such as high intraspecific variability in total egg count, and potential and absolute fecundity could also, in part, limit spawning success. The multispawning capacity of smallmouth bass was observed and demonstrated confirming earlier studies (Inslee, 1975; Cantin, 1988). Inslee (1975) reported that the total number of spawnings per female varied between one and eight in a southern population. For northern populations, one to four spawnings correlates with the number of clutches developing in the ovary, confirming other studies of Surber (1943), Beeman (1924) and Cantin & Bromage (1991). Other fish, for example European seabass, (Mayer *et al.*, 1988) are characterized by fractional spawning where successive clutches are spawned in quick succession; this may be for logistic reasons because of the high fecundity of seabass. Smallmouth bass by comparison have low fecundities. While egg releases have been reported to be successive over one (Langlois, 1932) to 36 hours (Beeman, 1924), individual females have been observed to release eggs over a few hours with a second spawning at two weeks to a month interval (Cantin, 1988). This would indicate fractional successive spawnings. The capacity of fast response coupled with the capacity of delaying it might become an evolutionary advantage in an unforseeable environment, particularly where temperature is concerned.

The information available in the literature combined with the data presented here clearly indicate that smallmouth bass spawning is temperature dependent; the smallmouth bass has the potential to be a unispawner or a multispawner and/or to resorb its oocytes based on physico-chemical parameters. The selection of a unispawning or multispawning strategy may be determined by the bass's perception of environmental conditions. Lambert (1984) and Lambert & Ware (1984) in their study on the strategy for reproduction of herring postulated that a succession of larval cohorts would spread spawning effort over time to take advantage of or counteract environmental variability. Lobon-Cervia *et al.* (1991) reported that single vs. multiple spawning could be an effect of summer droughts. Den Boer's (1968) suggested that spreading the risk in time can contribute to the stability of population size. Smallmouth bass could take advantage of the appropriate temperature conditions by using both reproductive strategies to assure

reproductive success in an unpredictable environment. The ability of smallmouth bass to employ single or multiple spawnings in response to environmental variations has not been reported before but could explain apparent contradictions in the literature about the number of spawnings and the number of eggs left in the ovary after spawning. This reproductive strategy is not unique among multispawners and would merit further investigation.

Unispawning or multispawning patterns have been found in different individuals raising the question of whether the same individuals change their strategy depending of environmental factors or do they remain as one type for their entire reproductive life span? The histological sectioning showed that constant conditions desynchronized ovarian development to the point of, in a few cases, finding oocytes at all stages without clear-cut differences between batches (Chapter 4 & 6). Histology did not permit the follow-up of the same individual; however, it indicated that different temperature-photoperiod conditions can lead to different patterns of development. This would suggest that smallmouth bass has a capacity to modify its spawning strategy in response to changes in its environment. Maintaining an array of strategies could obviously help compensate for the relatively low fecundity and year-to-year variability in hatching success and larval survival and display an individually integrated way to optimize reproductive success. This reproductive strategy has been related by Lebeau *et al.* (1986) to an adaptative strategy for production of a large number of eggs. Mann *et al.* (1984) suggested a phenotypic flexibility in response to geographical variation. A similar adaptability in terms of ovarian development, was suggested by Kestemont & Philippart (1991) for barbels kept in different environments. Smallmouth bass could be another example of plasticity in teleost reproductive tactics.

Lunar synchronization is a common occurrence among many marine tropical and temperate fish species (Johannes, 1978; Taylor, 1984; Schwanck, 1987; Garcia, 1992) generally associated with tidal cycles (Gibson, 1992). However, reproductive activity of freshwater fishes rarely follows the lunar cycle (Schwanck, 1987); only few cichlid fish have shown lunar synchronization (Nakai *et al.*, 1990). In these cases, two adaptative

advantages of lunar spawning cycles have been suggested: (1) moonlight provides a cue for the pairs to spawn at the same time, and (2) spawning prior to a full moon enhances the effectiveness of nocturnal parental care. While these suggestions might well apply to smallmouth bass, the observations of a higher rate of lunar synchronization associated with constant conditions could be considered a response to a non-cyclic environment in spite of the observed ovarian desynchronization (Chap. 3). These observations confirm Scott's (1979) speculation that the lunar cycle could be used as a synchronizer for populations in freshwater environments where coordinated spawning occurred in the absence of any other reliable synchronizing cues. The effects of the synchronizing cues are discussed in Chapter 6.

SUMMARY

Natural spawning of smallmouth bass occurred in May-June at 45°N 71°W latitude. Manipulation of temperature and photoperiod have permitted spawning most months of the year. The same female can spawn once or more than once; this plasticity in reproductive tactics can be a response to a variable environment. Ovarian desynchronization (batches of oocytes without clear-cut differences between stages) was related to constant conditions and lunar synchronization appeared more important under constant than natural conditions.

CHAPTER 4

MORPHOLOGY AND HISTOLOGY OF THE OVARY

4.1 Introduction

Morphology and histology of the ovary through macroscopic and microscopic observations have been used to define the yearly reproductive cycle of mature smallmouth bass maintained under natural, constant summer and winter conditions. Oocyte developmental patterns and reproductive cycles have been described for many teleostean fish (see review by de Vlaming, 1983); the patterns and cycles are mostly related to field observations on natural spawning (Coble, 1975; Inslee, 1975). There is limited information on smallmouth bass ovarian development (Cantin, 1988).

The terminology used in the literature to describe the ovarian/oocyte development is not standardized with respect to whether it is physiologically or histologically or species oriented. The present description of the oocyte stages and the yearly cycle of the smallmouth bass ovary was based on histological and *in vivo* monitoring of oocytes (Chapter 2). The ovarian/oocyte developmental pattern describing smallmouth bass growth phases comprised those defined for salmonid and non-salmonid fishes.

Atresia has been regarded as an uncommon phenomenon in healthy well-fed fish (de Vlaming, 1983) but was commonly observed in trout, accounting for 5-10% of the total number of oocytes (Bromage & Cumaranatunga, 1987). This phenomenon has been examined in detail because of its high incidence in this study.

4.2 Morphology of the ovary and its oocytes

The reproductive system of smallmouth bass consists of a paired ovary connected to the urogenital opening by an oviduct and supported in the body cavity by a dorsolateral fold of the peritoneum. The macroscopic appearance of the ovaries changes with the approach of spawning time. The ovary, in non-spawning periods are thin pinkish elongated structures with a slight granular appearance during previtellogenic phases.

They become opaque yellowish, during vitellogenic phases, to transparent with a yellow animal pole clearly visible at ovulation. The enlarged ovaries, in adult fish, measure 2-5 cm in diameter and 4-10 cm in length (Fig. 4.1), weigh 25-160 g and constitute up to 10-15 percent of body weight at spawning. The ovary is composed of an outer envelope, the ovarian wall, from which invaginates ovigerous lamellae where the oocytes can develop (Fig. 4.2). Clutches of oocytes at different stages are developing within these ovigerous folds, leaving spaces between the folds, the lumen. The lumen can receive the post-ovulatory oocytes before their expulsion through the urogenital opening at spawning. Mature oocytes can also be released by rupture of the ovarian wall.

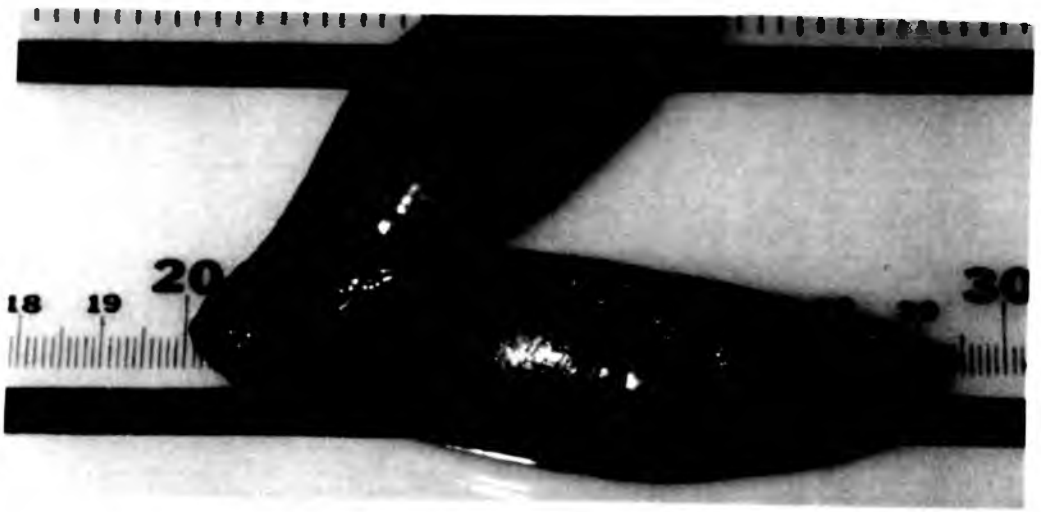


Fig. 4.1 The ovaries of smallmouth bass during the prespawning period (May) under natural conditions

Ovarian asymmetry in size was also noted. In 83% of the fish, the left ovary weighed up to 56% more than the right one. Asymmetry, in size of the ovaries, is typical of most birds and has been reported in reptiles and in some fish (Franchi *et al.*, 1962).

The ovary of smallmouth bass, based on Wallace & Selman's classification (1981), can be considered group synchronous, because more than two populations of oocytes from different developmental stages are present at any time. However, the observation of non-homogenous clutches of oocytes in the ovary under certain conditions and the

presence of all stages in the ovary at other times could suggest that the development pattern could also be asynchronous. This was the case, particularly, when data from the left ovary used for fecundity assessment and that from the right ovary used for histology were combined (Section 4.4).

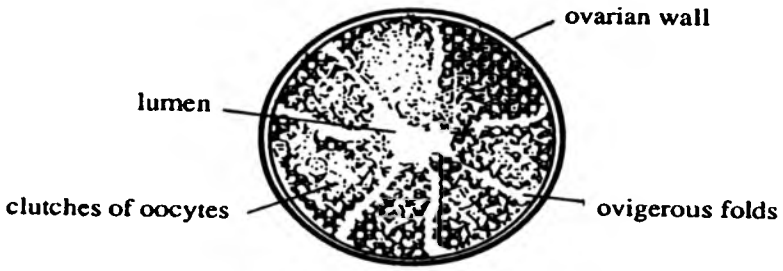


Fig. 4.2 Schematic of the ovary of smallmouth bass in sagittal view.

Observations with a dissecting microscope of the preserved oogonia/oocytes from ovarian catheterization showed, the oogonia as a slightly ovoid structure, white opaque, less than 5 µm in diameter and incorporated or associated with the ovarian stroma and/or the post-atretic follicle. Transformation of the oogonia into the perinucleolar oocytes is characterized by an enlargement of the cytoplasm. The previtellogenic stages (Fig. 4.3), the early and late perinucleolar, are transparent oocytes of an irregular shape, ranging from 25 µm to 300 µm. As they reach the end of the late perinucleolar stage, they appear less transparent and loose progressively their tight contact with the ovarian stroma. During the secondary growth phase (Fig. 4.3), the oocytes become slightly white opaque, and the vesicle stage measures between 350-700 µm; the vitellogenic oocytes become yellow and progressively loose their original opacity as the yolk globules fuse together. Their sizes range from 750 µm to 1700 µm in diameter. In tertiary growth (Fig. 4.3), maturing oocytes become more transparent, and the yolk globule, now completely fused, migrates to the animal pole, and is highly visible. The oocytes now measure 1700 µm to 2450 µm depending of the degree of hydration. Post-ovulated follicles and atretic oocytes (Fig. 4.3) were also observed but could not be staged accurately.

Fig. 4.3 Oocytes collected by catheterization, from left to right A: previtellogenic, B: early vitellogenic, C: late vitellogenic, D: mature, E: atretic, F: eggs

4.3 Ovarian histology

4.3.1 General description of the oocyte development pattern in teleosts

In marine teleosts, Barr (1968) divided the ovarian development into four phases: (1) oogonial proliferation by mitotic division, (2) oogenesis or meiotic transformation of oogonia into primary oocytes, (3) oocyte growth, and (4) maturation. Oogenesis has also been used (Raven, 1961; Wallace, 1985) to encompass the entire growth phase from oogonia through maturation. Bromage & Cumaranatunga (1988) however, recommended reserving the term oogenesis for the transformation of secondary oogonia into primary oocytes confirming de Vlaming's (1983) description. More recently, oocyte growth has been combined with vitellogenesis. Since Pan *et al.* (1969), in their study of female-specific bloodborn yolk precursors in insects, proposed the name vitellogenin for the lipoglycophosphoprotein complex, the process of oocyte incorporation of vitellogenin has acquired full recognition. Vitellogenesis (reviews by Ng & Idler, 1983; de Vlaming, 1983; Wallace, 1978, 1985; Ho, 1991) encompasses the process of protein yolk synthesis where oestrogen produced by the ovary in response to gonadotropin is introduced into the bloodstream to regulate the hepatic synthesis and secretion of vitellogenin. Also, as a response to gonadotropin, vitellogenin in turn is selectively sequestered, from the bloodstream, by growing oocytes and is proteolytically processed within the oocyte into accruing yolk proteins, of which lipovitellin and phosvitin are the most studied so far (Wallace, 1985). Vitellogenin uptake is by receptor mediated micropinocytosis (Redding & Patino, 1993); in the

rainbow trout, gonadotropin (GtH I) possibly induces receptor formation (Tyler, 1991). Thus ovarian sequestration of vitellogenin secreted by the liver is predominantly responsible for oocyte growth.

In seabass, Mayer *et al.* (1988) subdivided oocyte growth into two distinct phases: a primary growth phase (PGP) (Wallace & Selman, 1981), which is gonadotropin-independent (Khoo, 1979) and a secondary growth phase (SGP), which is gonadotropin-dependent. The PGP involves nuclear changes (Tokarz, 1978) and includes oogonial proliferation, chromatin nucleus stage (primary oocyte, 20-30 µm), early perinucleolus stage (apparition of the primordial follicle cells, 30-60 µm) and late perinucleolus stage (resting oocytes, 60-120 µm). During the perinucleolus stages, juxtanuclear masses, the Balbiani bodies, appeared as basophilic aggregates on histological sections. These are composed of various cellular organelles such as mitochondria, Golgi bodies, smooth endoplasmic reticulum, multivesicular bodies and lipid granules (Guraya, 1979). While this description applied to animal oocytes in general, Wallace & Selman (1981) described Balbiani bodies for teleosteans and Beams & Kessel (1973) described them for rainbow trout. However, Mayer *et al.* (1988), in their study of seabass ovaries, showed the Balbiani bodies as unique crescent-shape structures, with a dual nature of basophilic and non-basophilic components, which broke down and dispersed before the secondary growth phase.

The secondary growth phase (SGP), for seabass, involves deposition of yolk, *i.e.* vitellogenesis divided into vesicle stage I, 110-160 µm and II, 155-250 µm, (appearance of small vesicles containing unsaturated lipids and the zona radiata, respectively), and a yolk granule stage, primary (260-440 µm), secondary (430-530 µm) and tertiary (530-800 µm) characterized by an increase in size and number of protein yolk granules. The third oocyte developmental stage, maturation, corresponded to the resumption of meiosis in the postovulatory oocyte with the migration and dissolution of the nucleus followed by the coalescence of the protein yolk granules and lipid yolk droplets. Hydration of the oocytes resulted in an increase of the volume and a translucent appearence. A percentage of postvitellogenic oocytes which failed to

undergo maturation/ovulation became atretic, then degenerated and were resorbed by active phagocytic granulosa cells.

Histochemistry of goldfish oocytes during their vitellogenic stages showed a clear distinction between the yolk vesicles and the yolk granules differentiated by their type of yolk inclusions (Khoo, 1979). Furthermore, three types of yolk, vesicles, granules and globules were observed in the smelt (Yamamoto, 1956).

The stages of oocytes development in trout, (Bromage & Cumaranatunga, 1988) followed the same pattern, as the seabass, with a distinction between oogenesis and folliculogenesis and a different terminology under the secondary growth with the exogenous vitellogenic stages including peripheral yolk granule and yolk granule migration stage which has not been described in seabass (Mayer *et al.*, 1988). Near the end of oogenesis, the vitelline envelope (the acellular structure) started to exhibit ultrastructural changes (Dumont & Brummett, 1985). This layer thickened and was penetrated by microvilli from the oocyte and by processes from the granulosa cells and the follicle layer. In non-mammalian vertebrates, this cellular layer is invested by a basal lamina and a collagenous matrix through which a vascular network courses (Wallace, 1985). This matrix is frequently termed the zona radiata.

Atresia can be described as the process (es) whereby oocytes are lost from the ovary other than by ovulation. It is a degenerative process that can occur at any stage of oocyte development. The description used for smallmouth bass was based on the works of Khoo (1975), Cumaranatunga (1988) and Hunter & Macewicz (1985) and considered oocyte (preovulatory or *corpora atretica*) and follicular atresia as two possibly different processes. Oocyte and follicular atresia can occur simultaneously and/or independently and may have a different fate.

In the literature, atresia has generally been divided into five stages of pre- and post-vitellogenic reabsorption, the alpha (α), the beta (β), the gamma (γ), the delta (δ) and the epsilon (ε) stage(Khoo, 1975). Other authors, Hunter & Macewicz (1985) have

divided atresia differently with an α stage and three follicular atretic stages. Strictly speaking, *corpora lutea* are normally formed from all ruptured follicles and can act as post-ovulatory endocrine structures; in case of pre-ovulatory atresia, where the follicles have not ruptured, the terms *corpora atretica* or follicular atresia seem more appropriate. Preovulatory "*corpora lutea*" are common in teleost ovaries (Browning, 1973); however there is limited information on their endocrine role. A transient (hours to several days) teleostean postovulatory *corpora lutea* has been reported in many ovoviviparous teleosts (Jones & Baxter, 1991). The luteal (granulosa) cells of these follicles have many similarities with the mammalian luteal cells and can last longer in teleosts with complex nesting behaviour (Jones & Baxter, 1991). The endocrine function of *corpora lutea* in oviparous teleosts remains enigmatic, although Liley *et al.* (1986) proposed a role of luteal steroids in spawning behaviour. Because only the ε stage could be associated with the presence of lutein pigments and oogonia, it was differentiated from the other stages considered as *corpora atretica*.

The description by Hunter & Macewicz (1985) of atresia in the anchovy ovary characterized the α stage of atresia by the disintegration of both the nucleus and the yolk globules which became less regular in shape. Following these changes, the zona radiata slowly dissolved whilst the granulosa cells became enlarged and invaded the degenerating oocyte as the zona radiata disappeared. The yolk globules became phagocytosed by the adjacent granulosa (luteal) cells. This stage persisted until the cytoplasm and the yolk were reabsorbed. The process took longer for post-vitellogenic oocyte than for previtellogenic oocytes, making it difficult to observe early atresia in this last group. They considered the α stage as the only one for oocyte degeneration. Khoo (1975) looked at the morphological changes of atretic oocytes in goldfish, and described the first stage mainly as an hypertrophy of the granulosa cells and a rupture of the yolk vesicles.

In the anchovy, the β stage is characterized by a compact structure composed of disorganized granulosa cells with occasionally one or more intercellular cavities. The follicles may then be completely reabsorbed or continue into the γ and δ stages or go

directly to the δ stage. In the goldfish, the β stage corresponds to the main invasion of the cytoplasm by the granulosa cells with digestion and resorption of the yolk inclusions.

The γ stage of atretic follicle in anchovy shows a flocculent material of light-yellow hue and fewer thecal cells while in goldfish it is described as a complete resorbtion of the oocyte leaving an hypertrophied granulosa layer surrounding a central cavity.

The δ stage, the last atretic stage described for the anchovy is characterized by the presence of a yellow-brown pigment in the granulosa cells and the disappearence of the thecal cells. No mention of nests of oogonia are made. In the goldfish, during this stage, the granulosa cells collapsed to form an irregular cellular mass and yellow-lutein pigments were observed amongst the cells. This ε stage appeared to differentiate into oogonia while yellow-lutein pigments were still visible. Histogenesis of the goldfish *corpus luteum* was divided into three stages morphologically similar to stage γ, δ and ε of the pre-ovulatory oocytes, respectively.

4.3.2 General description of the oocyte development pattern in smallmouth bass

4.3.2.1 Oogenesis and primary growth of oocytes

Oogonia are small rounded cells with a high nucleus to cytoplasm ratio (de Vlaming, 1983). Like for many other teleosts, oogonia occur in nests (Fig. 4.4), associated with yellow-brownish lutein pigments after haematoxylin-eosin staining (Fig. 4.5). The nests appear irregular in shape and highly vascularized (Fig. 4.6) indicative of intense metabolic activities. Nests of oogonia are quite often found in spaces left by ovulated and/or degenerated oocytes near yellow-brown pigments associated with the ε stage.

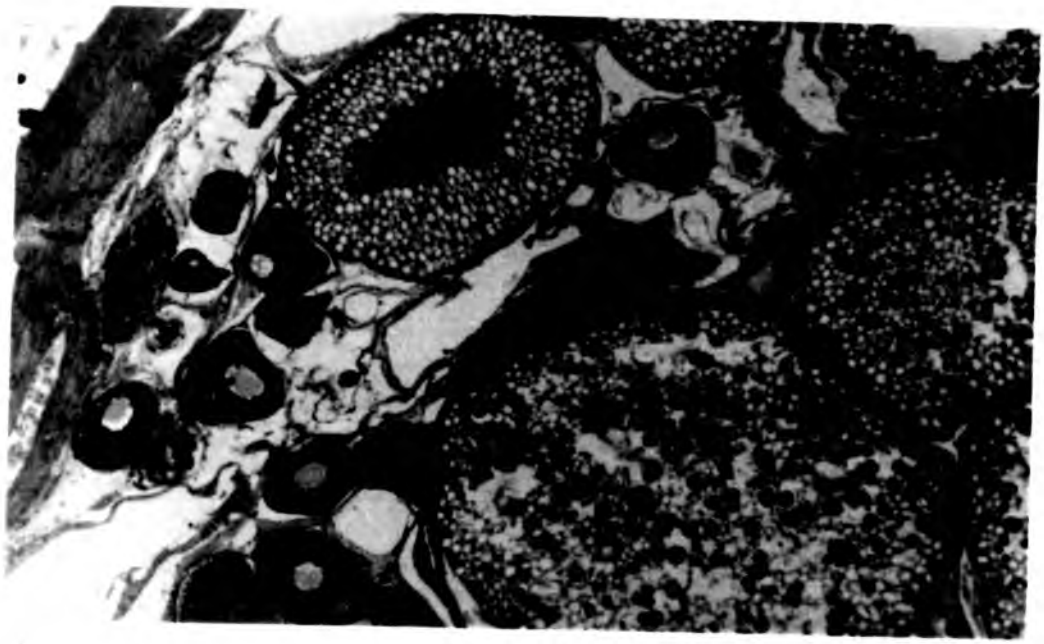


Fig 4 4 Section of an ovary showing nests of oogonia (og) with previtellogenic and vitellogenic oocytes. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

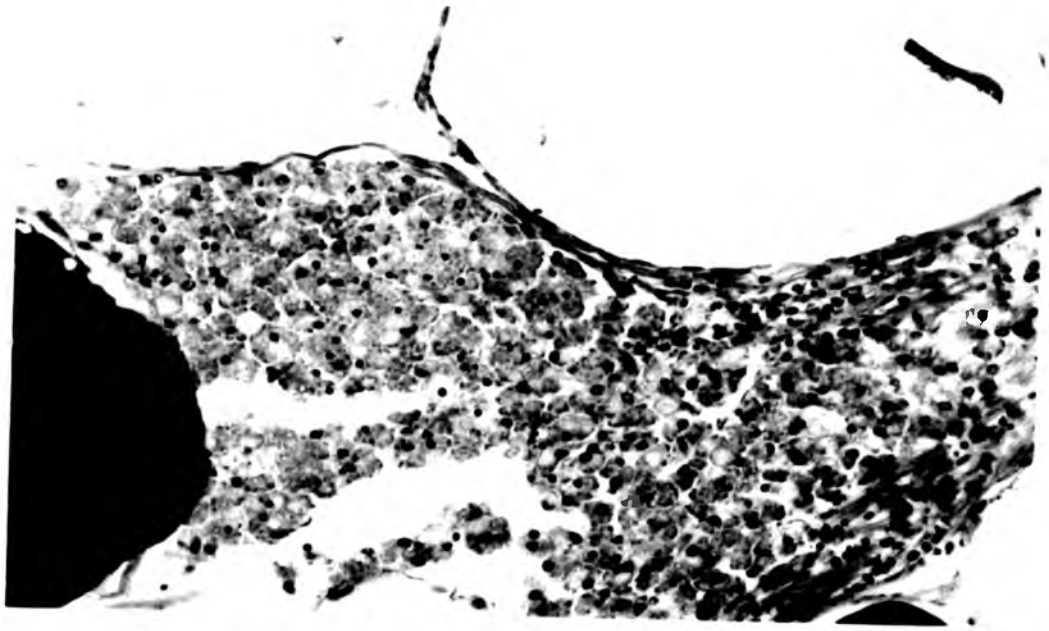


Fig. 4.5 Proliferation of oogonia during primary growth associated with yellow-brownish lutein pigments after Harris' haematoxylin and eosin staining. Scale: 1 cm = 0.023 mm

Oogonia are most often present in pre- and post-vitellogenic ovaries where atresia is also observed. Oogonial proliferation (mitotic division) appears to occur cyclically as reported for other fish with group synchronous type ovary (Franchi *et al.*, 1962; Bromage & Cumaranatunga, 1987).

The haematoxylin uptake by the oocyte, in the early perinucleolar stage, is indicative of the preponderance of nuclear material. Early and late perinucleolar stages are characterized by the presence of multiple nucleoli located at the periphery of the nucleus. The early perinucleolar oocyte has an initial diameter of 25 µ. Early growth leads to a ten fold increase in size and less deep staining with the haematoxylin. The final diameter, by the late perinucleolar stages, can be up to 15 times greater than the initial one. During this primary growth phase, the nuclear-to-cytoplasm ratio decreases as the oocyte volume increases. Perinucleolar oocytes often reflect a satellite organization around an older oocyte (Fig. 4.7). Early perinucleolar stages were not found in all fish investigated, but late perinucleoli were always present.

Small white vacuoles were found in the cytoplasm of early and late perinucleolar oocytes, starting from the periphery and progressively moving toward the nucleus as their number increased (Fig. 4.8 & 4.9). These vacuoles which did not stain with haematoxylin-eosin could be an artifact of the cortical alveoli. They first appear whilst the oocyte is still staining deeply with the haematoxylin during the perinucleolar stage. The transition between the pre- and vitellogenic stages is less well defined than what was described previously for other species. The presence of basophilic aggregates, that could correspond to a non-homogenous structure composed of various cellular organelles appearing as a darkly stained irregular structure within the cytoplasm, was observed in some oocytes. These structures are called "Balbiani bodies"; however, they do not resemble, either in size or shape, the structures described earlier by Mayer *et al.* (1988). Compared to early perinucleolar stage, the late perinucleolar stage (resting stage) was enlarged and stained less deeply with haematoxylin.

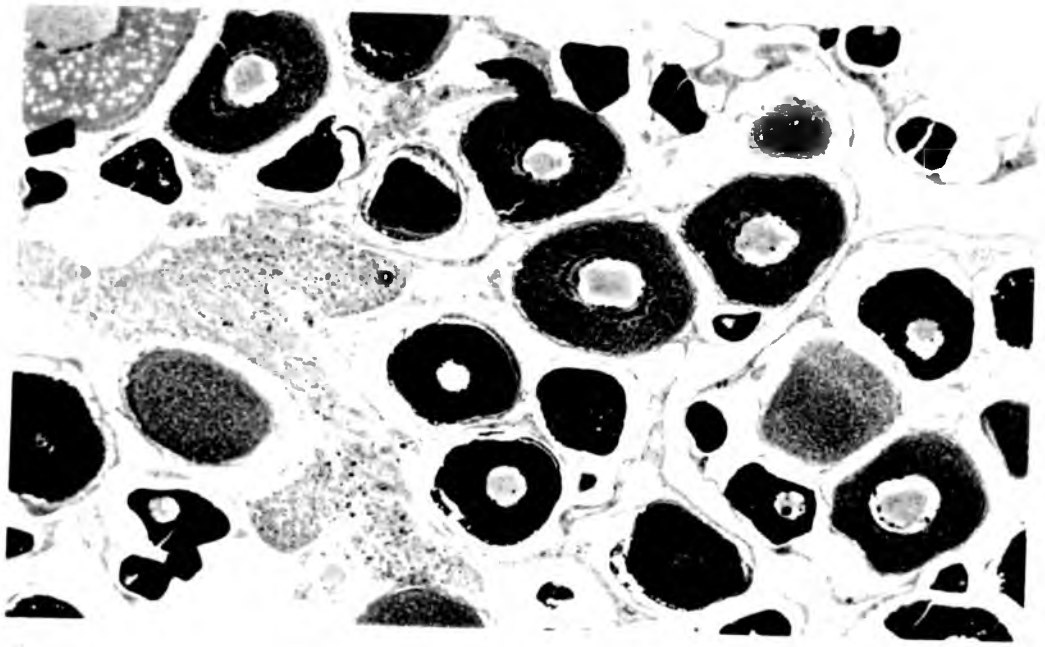


Fig. 4.6 Vascularization and oogonial proliferation stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

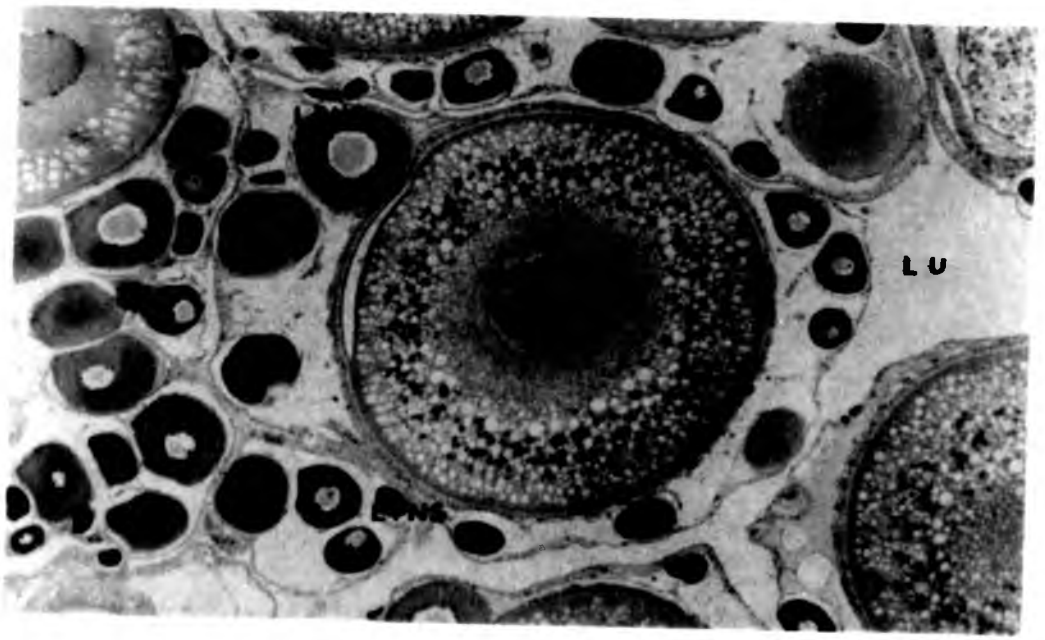


Fig. 4.7 Satellite organization of perinucleolar oocytes, early (EPNS) and late (LPNS) around an early vitellogenic oocyte. The lumen (LU) of the ovary is also shown. Scale: 1 cm = 0.22 mm

At the end of primary growth, a single follicular layer starts to encapsulate the oocyte and to form the granulosa layer (Fig. 4.9). The teleost follicle comprises an oocyte surrounded by cellular epithelial cells or the granulosa and can behave, to some extent, as an independent physiological unit (Jalabert *et al.*, 1991). The granulosa is usually formed by a single layer of cells. External to the granulosa is a layer of connective tissue cells, the theca. It consists of a layer of flattened fibroblast cells which normally first appear at the end of the perinucleolar stage of the primary growth phase (Forberg, 1982). The thecal cells are closely associated by a rich capillary plexus to the granulosa. The zona radiata, which first appears at the end of the primary growth phase, is secreted between the oocyte and the granulosa. In oviparous teleosts, it normally consists of 3 distinct layers differing in structure and possibly origin, into which villi from both the oocyte and granulosa cells project (Dodd & Sumpter, 1983). The formation of the follicular envelope marks a physiological transition where the oocyte can respond to its environment and the beginning of the secondary growth phase.

4.3.2.2 Secondary growth and vitellogenesis

This phase is normally characterized by the development of a chorionic membrane (zona radiata), the presence of glycoprotein contained in the cortical alveoli (yolk vesicle) and the sequestration of a lipophosphoprotein (vitellogenin). The literature is confused about the use of "yolk vesicle" which can refer to the uptake of lipidic material and/or proteinaceous material. These vesicles are reported under many names including cortical alveoli, intravesicular yolk, vacuoles, vacuome, yolk globules, yolk spheres and yolk vesicles. While earlier work suggested that these vesicles contain an intravesicular yolk (Marza *et al*, 1937), there is little evidence that these vesicles are involved in any yolk processing (Bromage & Cumaranatunga, 1988). Khoo (1979) demonstrated, in the goldfish, that these vesicles contain mucopolysaccharides and proteinaceous material while the yolk granules contain a larger quantity of proteinaceous material, neutral fats and phospholipids. In smallmouth bass' sectioned ovaries, haematoxylin-eosin staining gave no colouration to these vesicles giving them

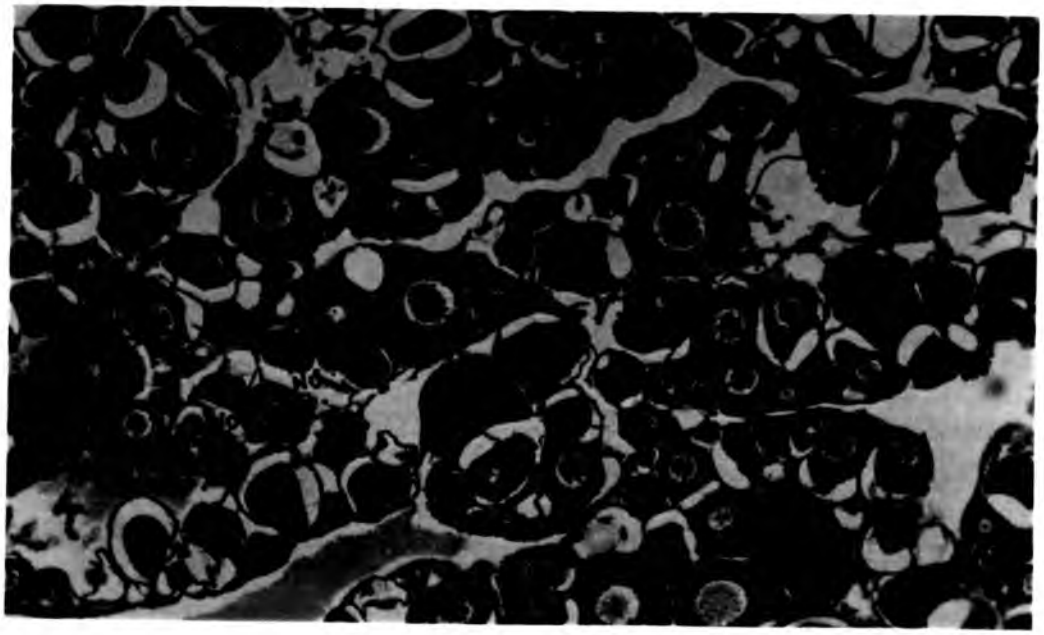


Fig. 4.8 Section of a previtellogenic ovary, showing early and late stages with the presence of the nucleus (NU), numerous nucleoli (NO). Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.09 mm

Fig. 4.9 Late perinucleolar oocyte with the nucleus (NU), the numerous nucleoli (NO) and the apparition of few vesicles. A follicular layer, the granulosa (luteal) is present (FL). The late perinucleolar oocytes stained less intensely with Harris' haematoxylin and eosin than the early. Scale: 1 cm = 0.02 mm

the look of empty vacuoles. The same artifact was reported by Khoo (1979) in the goldfish ovaries which could suggest a similar composition.

The first stage in secondary growth of oocytes corresponds to the vesicle or cortical alveoli stage where the "yolk vesicles", pushed to the periphery during late perinucleolar stage, have increased in number and size, and are dispersed toward the centre of the oocyte (Fig. 4.10). At this stage, the granulosa and the chorionic layers are present. The chorionic membrane also known as the *zona radiata*, *zona pellucida*, vitelline envelope, egg envelope or primary envelope is an extracellular layer which, in the trout, is produced by either the granulosa or oocyte or more likely a combination of both (Bromage & Cumaranatunga, 1988). It is acellular but is penetrated by pore canals which become the characteristic striations of the thicker *zona radiata* in subsequent stages. The cells of the granulosa layer become more cuboidal as the oocytes grow. The oocytes measured between 350 and 700 µm. This stage was normally present in ovaries of fish with more mature stages of development.

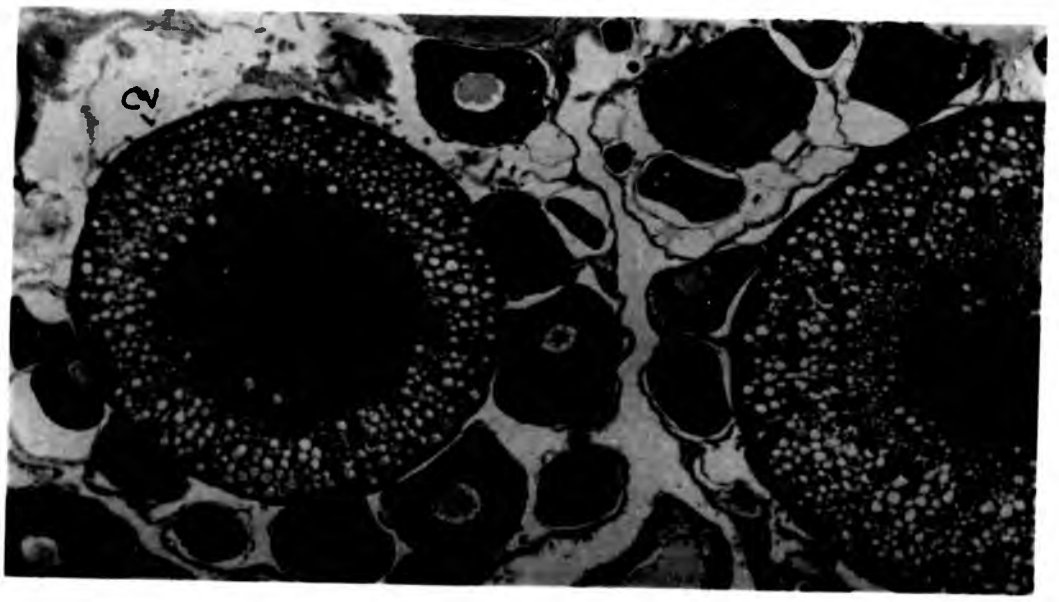


Fig. 4.10 Cortical vesicle (CV) stage with ooplasm filled with vesicles and the presence of the different follicular layers. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.09 mm

Small yolk granules which stain dark pink with haematoxylin and eosin appear progressively between the cortical alveoli, which form a large peripheral ring covering most of the oocyte (Fig. 4.11 & 4.12). In the rainbow trout, this yolk is derived from a 440,000 molecular weight precursor lipophosphoprotein-calcium complex called vitellogenin, which is synthesized by the liver, released in the blood and sequestered by the oocyte (Tyler *et al.*, 1987, 1990). Observations of these lipophosphoproteins as part of fish' yolk have been confirmed for different species (reviewed by Babin & Vernier, 1989). In smallmouth bass (Chapter 4), these yolk granules could be of similar origin.

The small yolk granules first appear like a ring around the nucleus then move toward the periphery of the oocyte to cover ultimately the entire ooplasm (Fig. 4.13 & 4.14). As the process of vitellogenesis continues, the yolk granules will coalesce to form yolk globules, which increase in size until they fill the entire oocyte (Fig. 4.15).

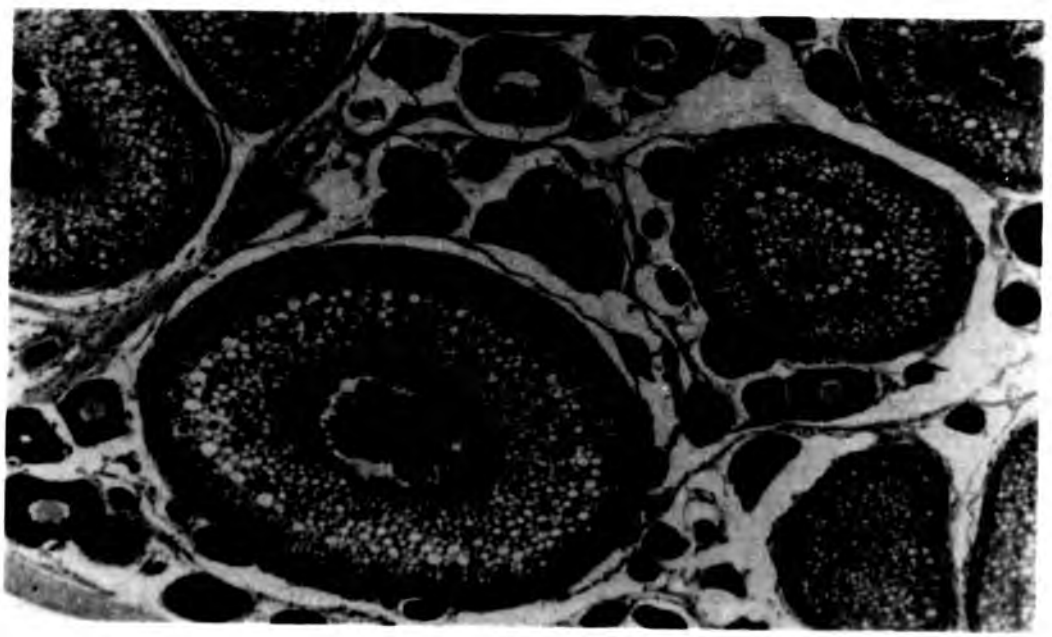


Fig. 4.11 Early vitellogenic oocyte characterized by the apparition of yolk granules (YG) between the vesicles (V), starting from the periphery. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

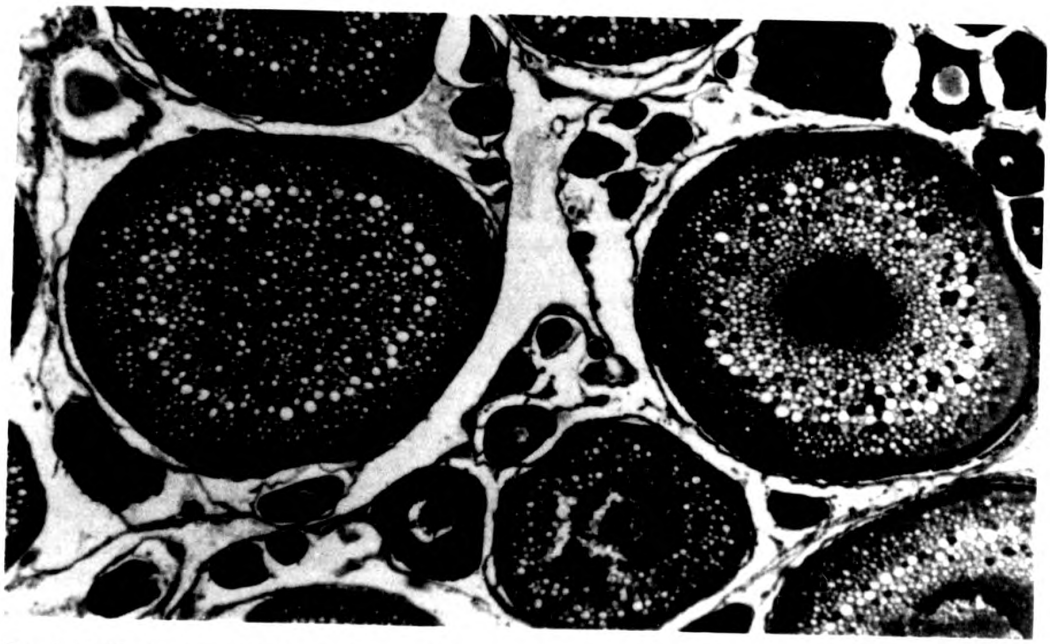


Fig. 4.12 Vitellogenic oocyte showing the progression of yolk granules toward the centre of the oocyte. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

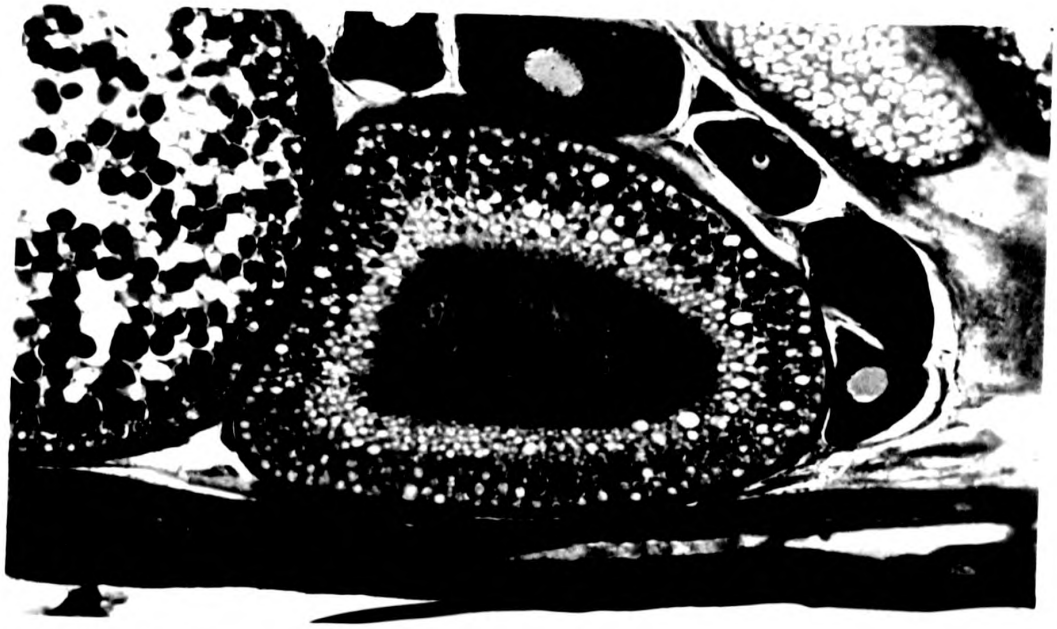


Fig. 4.13 Progression of vitellogenesis. Scale: 1 cm = 0.09 mm

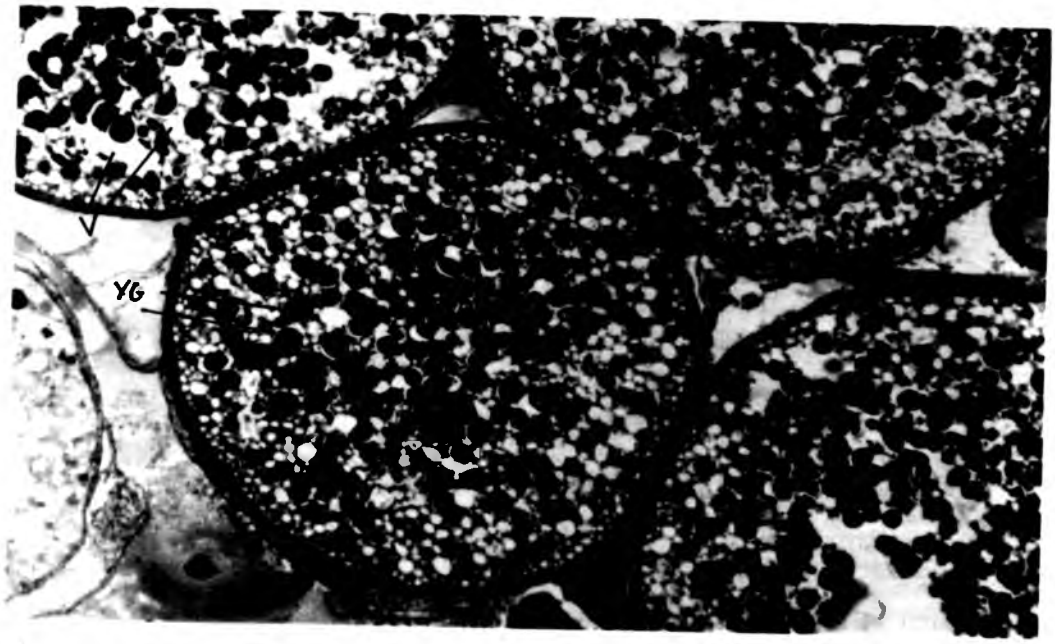


Fig. 4.14 Section of ovary during vitellogenesis showing the growth of the yolk granules (YG) as their multiplication, forcing the cortical vesicles (V) to coalesce. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

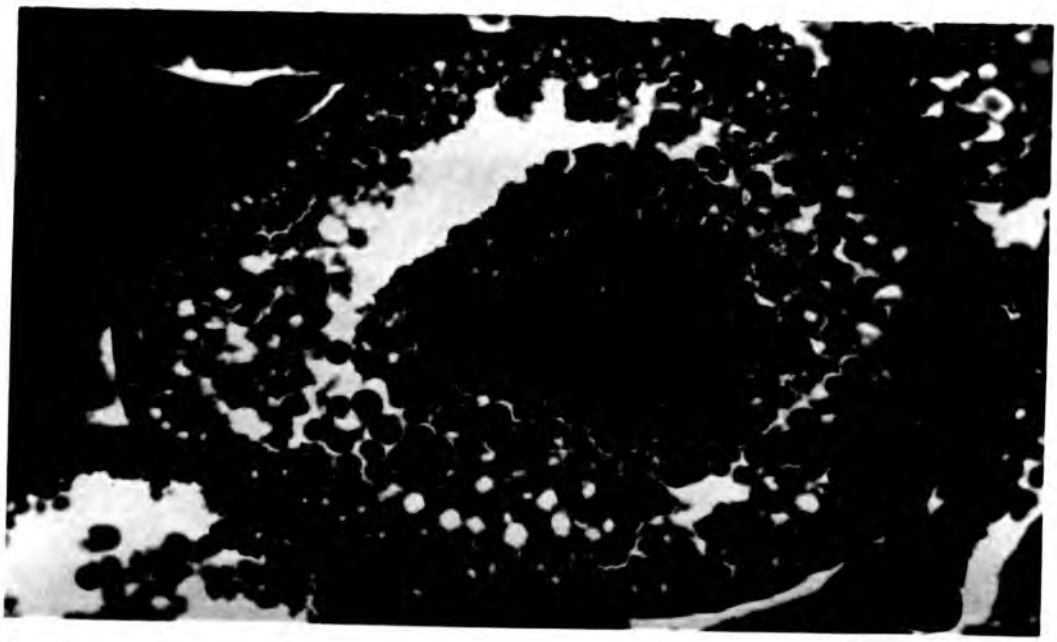


Fig. 4.15 Section of a maturing oocyte where the cortical vesicles have coalesced and the yolk granules have started to merge. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

Ultimately, they aggregate to fill the whole central area. Vitellogenesis, in smallmouth bass, started by the end of summer (end of August-early September) and oocytes slowly matured throughout winter. Vitellogenic oocytes may be subdivided into two stages, early vitellogenesis, with sizes ranging from 0.75-0.95 mm and vitellogenesis with oocytes from 1-1.7 mm. During vitellogenesis, the follicular layers (Fig. 4.16, 4.17 & 4.18) increase in size, particularly the zona radiata that becomes thicker with the striations more easily visible.

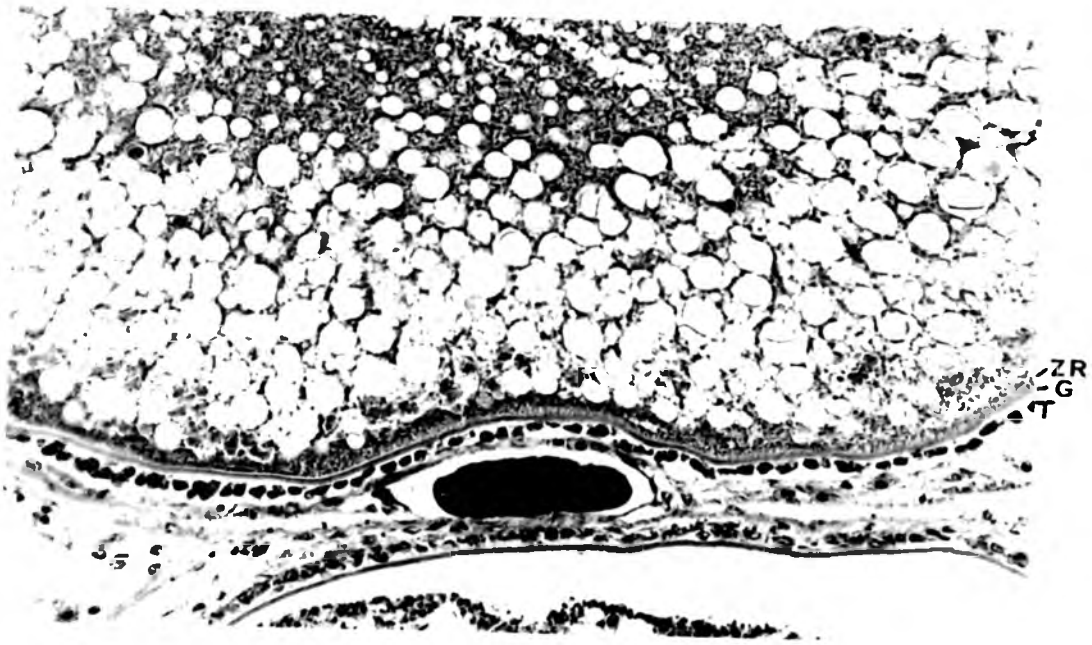


Fig. 4.16 Follicular layers during early vitellogenic phases, including from the exterior to the interior of the oocyte, the theca (T), the granulosa cells (G) and the zona radiata (ZR). Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.12 mm

The last stage before ovulation, oocyte maturation, involves coalescence of the protein yolk granules and the lipid yolk droplets (Fig. 4.19). The fusion of lipid yolk droplets and protein yolk granules appears similar to what Mayer *et al.* (1988) reported for sea bass. The oocytes rapidly increase in volume to reach 2.3-2.5 mm prior to ovulation and appear homogenous and translucent with a unique yolk globule which has migrated to one pole.

Fig. 4.17 Follicular layers during vitellogenic phases, including from the exterior to the interior of the oocyte, the theca (T), the granulosa cells (G) and the zona radiata (ZR). Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.09 mm

Fig. 4.18 Enlargement of follicular layers during late vitellogenic phases, including from the exterior to the interior of the oocyte, the theca (T), the granulosa cells (G) and the zona radiata (ZR). Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.023 mm

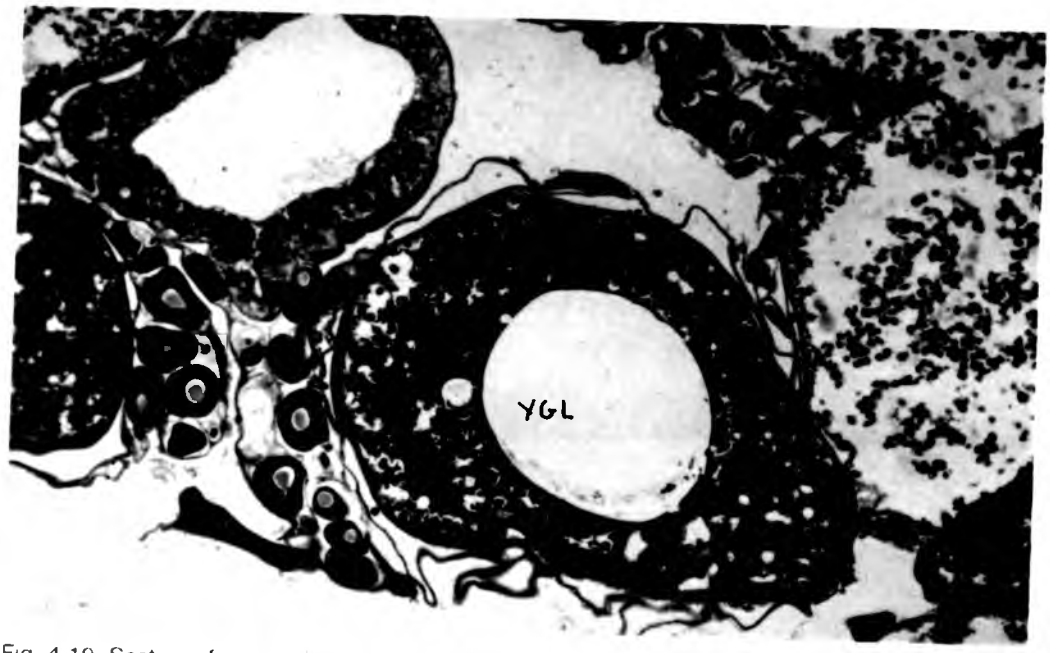


Fig. 4.19 Section of a maturing oocyte with its yolk globule (YGL) and the yolk granules fusing together. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.09 mm

4.3.2.3 Ovulation

Ovulation is the process by which the mature oocyte is expelled from its follicle into the lumen of the ovary and/or ruptures the ovarian wall to reach the peritoneal cavity (Fig. 4.20). It involves the rupture of the follicular layers and is triggered, in certain fish, by maturational steroids, which may induce the formation of specific prostaglandins, which, in turn, stimulate ovulation via calcium-mediated events (Goetz *et al.*, 1991). This process is normally closely related to the act of spawning preceding it by only a short period of time. If the fish is prevented from spawning, atresia will recycle the contents of oocytes and their follicle.

Ovulated oocytes are translucent compared to intrafollicular oocytes and contain only one lipid droplet at one pole. Ovulated oocytes were observed in fish maintained under natural conditions or during spawning. Their absence under the other conditions might be due to the elapsed time between sampling and/or the inability of smallmouth bass to ovulate in a crowded environment (Chapter 5).

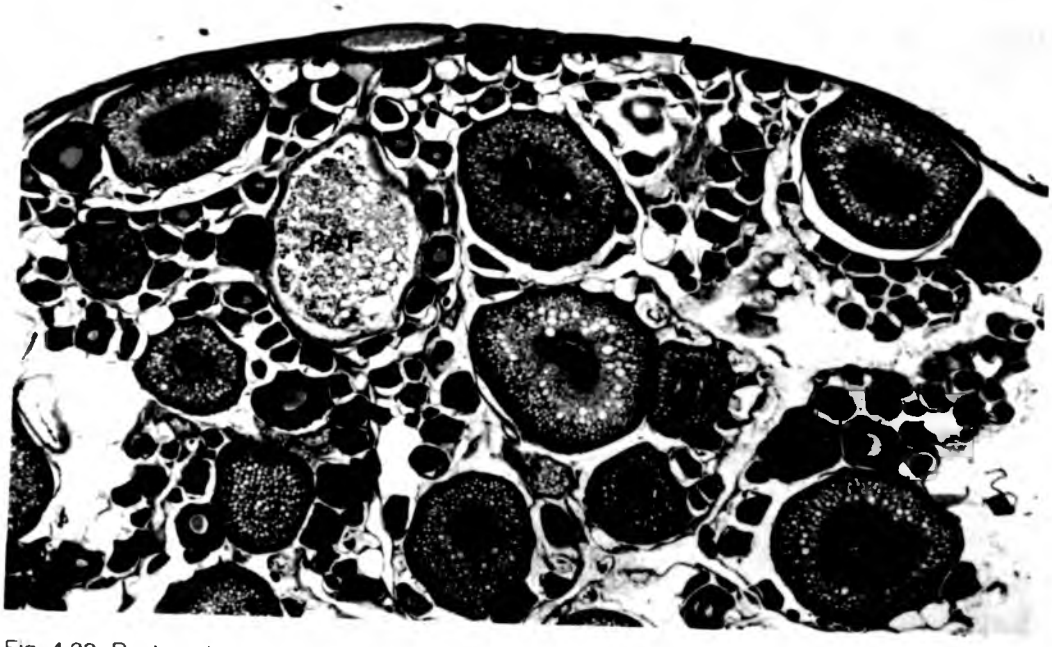


Fig. 4.20 Post-ovulatory follicle (POF). Scale: 1 cm = 0.22 mm

3.3.2.4 Atresia

For the purpose of this study, atresia was looked upon from two different perspectives: a degenerative process of pre- and post-vitellogenic oocytes and a modulating process for oocyte development. While all the stages described in the literature were observed in smallmouth bass ovaries, it remained very difficult to assess their pattern of resorption as the study was not specifically designed to assess atresia. Ingram (1962) pointed out that no one has followed the same atretic oocyte during its different stages. It became obvious that the fate of atretic oocytes was difficult to define with certainty. However, as this degenerative process accounted for up to 70% of the oocytes under constant winter conditions, stages and specific observations were reported. Atresia consists of several stages, α, β, δ, γ, ε, already described in Section 4.3.1.

In smallmouth bass, the α stage of atresia is morphologically characterized by a change of shape from an ovoid to a less regular shape and histologically, by the disappearance of the follicles integrity *i.e.* the granulosa layer lost its organization to become what Beach (1959) called "syncytial". The degeneration of yolk vesicles (Fig. 4.21 & 4.22)

started from the periphery toward the center, concentrating the yolk into a small mass and leading to the β stage. During the β stage, the oocyte inclusions and yolk vesicles seemed to be engulfed as the granulosa cells progressed in their invasion (Fig. 4.23). While the yolk was disintegrating and shrinking in size, the granulosa cells proliferated and hypertrophied to form a compact well vascularized structure termed the *corpus atreticum*, resorbing yolk and the follicle. The completion of the yolk's resorption was shown by the enlargement of the small empty cavity (Fig. 4.24), leaving the thick granulosa cells to their progressive regression during stage γ (Fig. 4.25). The follicles collapsed encapsulating what can be left of the oocyte material particularly the yellow-lutein pigments and their association with the appearance of nests of oogonia occurring during stage ε (Fig. 4.26). Post-ovulatory atresia could not be definitively assessed due to the fact that only smallmouth bass captured from natural environment or during the spawning season (May-June) included ovulated eggs. Or, as pointed out by Hunter & Macewicz (1985) if smallmouth bass oocyte development is similar to that of the anchovies, only the α stage can be easily distinguished from post-ovulatory follicles whereas this was not the case for later atretic stages. In any case, the β stage (Fig. 4.23) could indicate the presence of a pre-ovulatory *corpus luteum*.

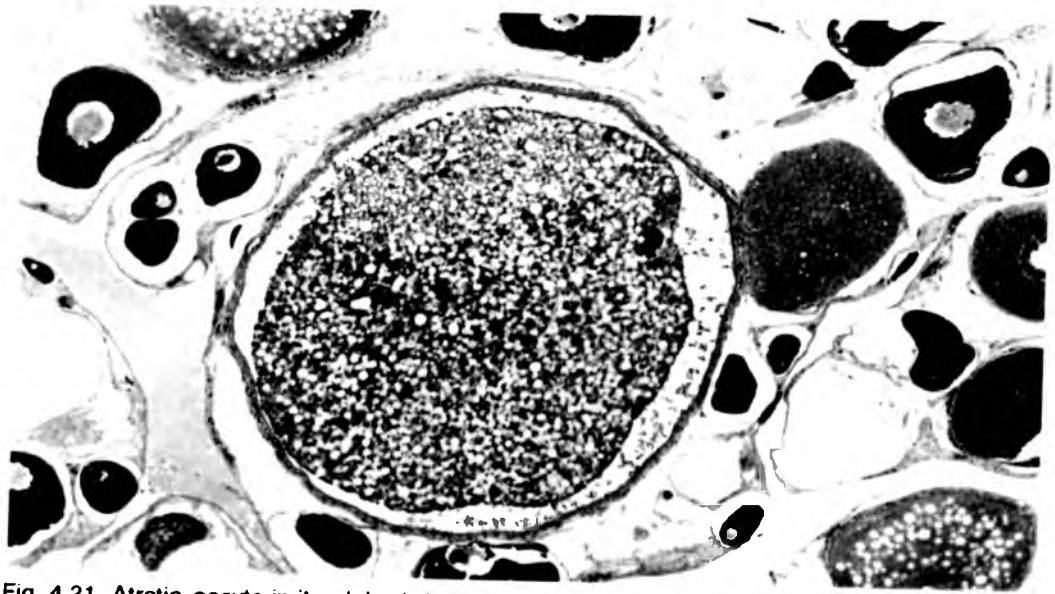


Fig. 4.21 Atretic oocyte in its alpha (α) stage showing the disintegration of the yolk granules and the early migration of the granulosa cells. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

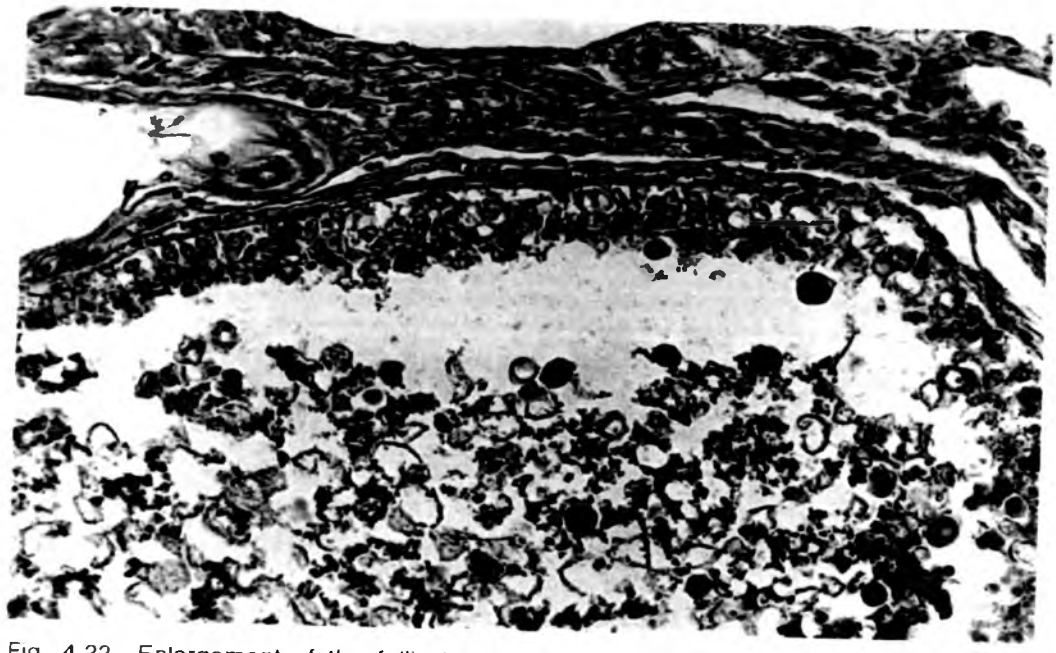


Fig. 4.22 Enlargement of the follicular layers during early atretic processes showing hypertrophy of the thecal cells. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.12 mm

Fig. 4.23 Atretic oocyte in their beta (β) stage showing oocyte and follicular atresia with invasion of granulosa cells digesting the suspended yolk. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.09 mm

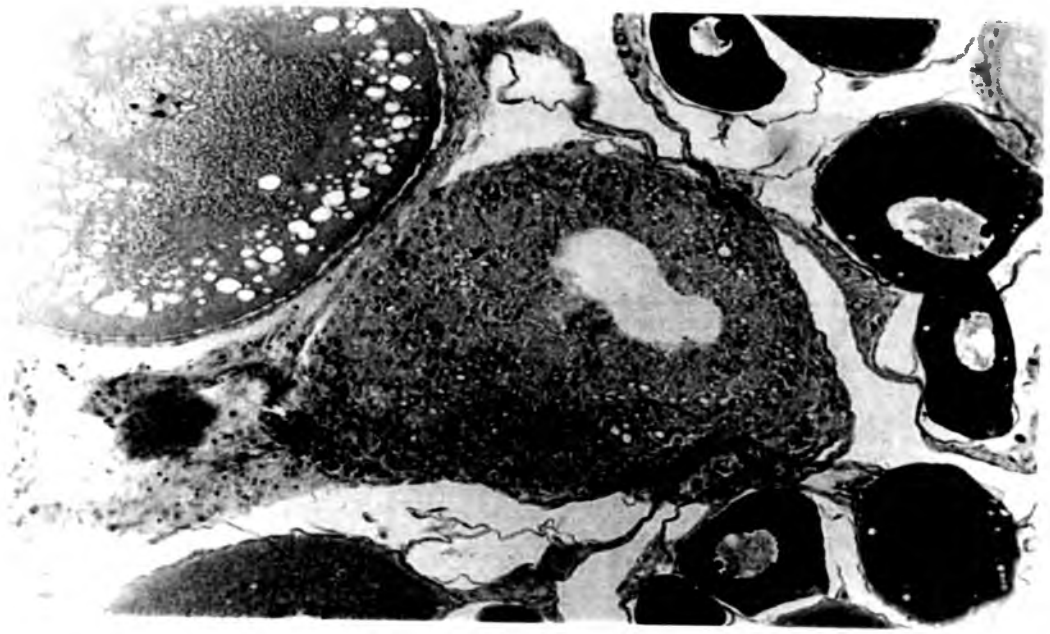


Fig 4.24 Atretic oocyte in their beta (β) stage showing oocyte and follicular atresia with invasion of granulosa cells digesting and resorbing the yolk by active phagocytosis. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.12 mm

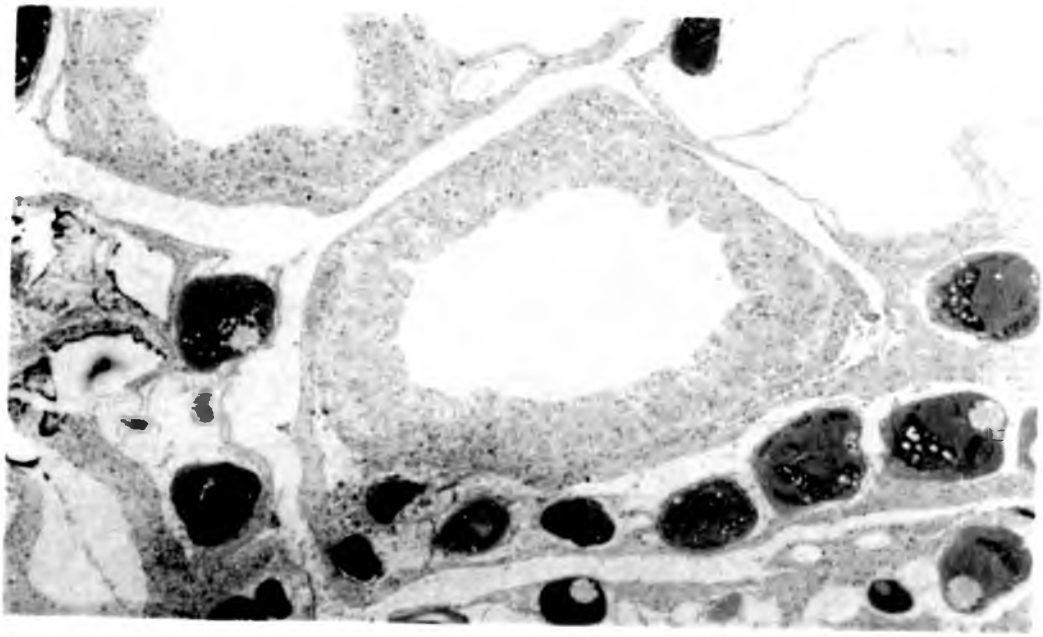


Fig. 4.25 Atretic oocytes in their gamma (γ) stage showing the remained follicular atresia with the progressive regression of granulosa cells towards the periphery. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.22 mm

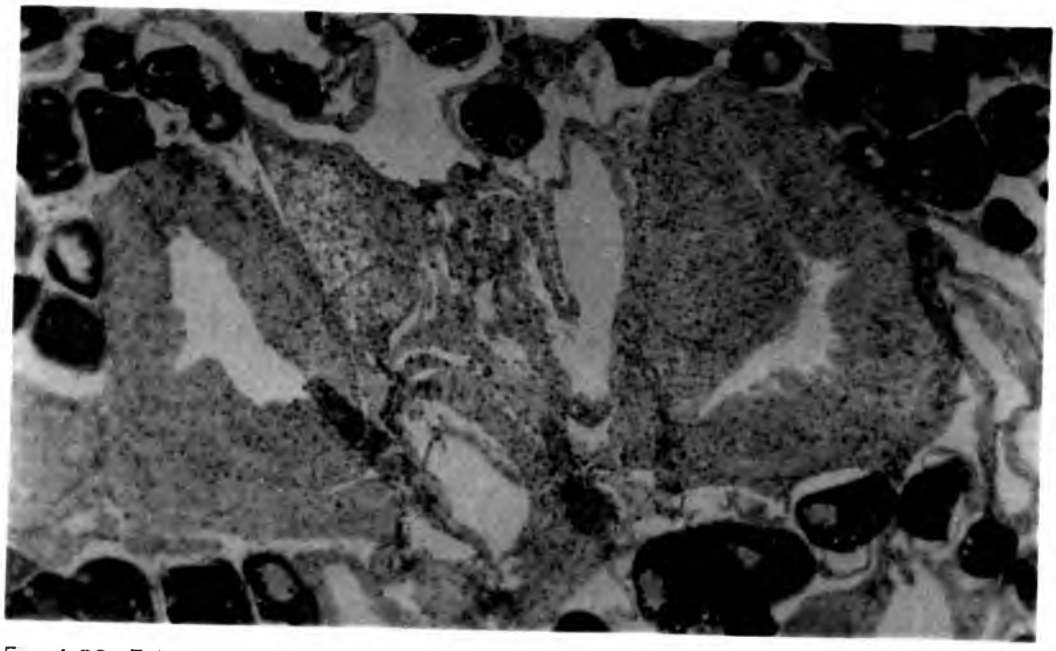


Fig. 4.26 Enlargement of the gamma (γ) stage showing the remained follicle collapsing. Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.09 mm

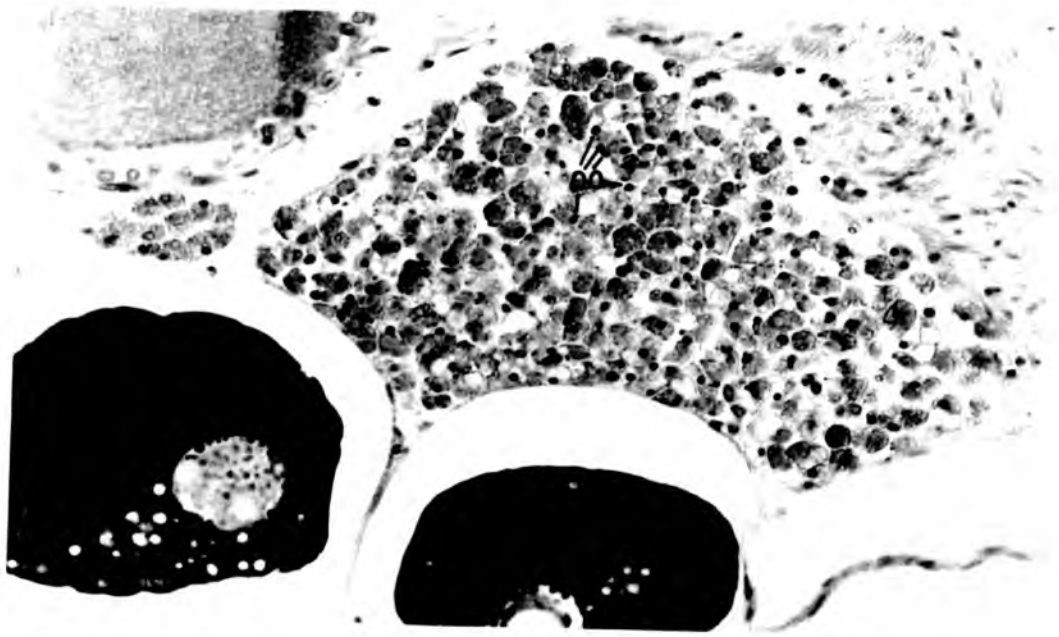


Fig. 4.27 Atretic oocyte in its epsilon (ε) stage showing the remained lutein pigments associated with nests of oogonia (OO). Stained with Harris' haematoxylin and eosin. Scale: 1 cm = 0.023 mm

4.4 Oocyte development pattern

There was a sequential progression, from the anterior to the posterior part of the ovary, as shown by sagittal sectioning (Fig. 4.28), in the development of oocyte stages leading to more advanced stages closer to the urogenital opening. These changes corresponded to a larger number of the more mature stages in the posterior part of the ovary. This observation has led to the use of the mid-section data from the histological work for comparison with the oocytes collected from the catheterization.

Data combined from the histology, the *in vivo* oocyte catheterization and the Gilson's preserved oocytes were used to establish the yearly cycle of ovarian/oocyte development in smallmouth bass. Primary data (Table 4.1) and a two tailed Student's-t test (Table 4.2) showed that the frequencies of vitellogenic and mature oocytes were not significantly different ($P>0.05$) between catheterization and Gilson's fluid methods, however, the frequencies of previtellogenic oocytes were significantly different ($P<0.05$). Early and late perinucleolar oocytes preserved in Gilson's fluid were not easily counted because of their transparent appearence under catheterization technique and their resemblance to small pieces of the broken ovarian walls. This could explain the discrepancy between the two techniques. There was no difference in size for all stages tested ($P>0.05$) between the two methods.

Ovarian catheterization was also used to monitor the pattern of individual ovarian development in groups A2, D2 and N2 (Table 2.2). Individual follow-up of oocyte development (Fig. 4.29a-j) under natural conditions indicated the simultaneous presence of different clutches of oocytes and a gradual transformation of previtellogenic oocytes, peaking in August, into vitellogenic oocytes during the autumn and the winter. Mature oocytes were present in the spring simultaneously with the decreasing number of vitellogenic oocytes. Previtellogenic and early vitellogenic stages prevailed under constant summer conditions and further vitellogenesis was limited. Previtellogenic stages were almost absent under constant winter conditions whilst vitellogenic, mature and atretic oocytes prevailed with atresia sporadically dominating. This would suggest a slow growth of vitellogenic oocytes to maintain

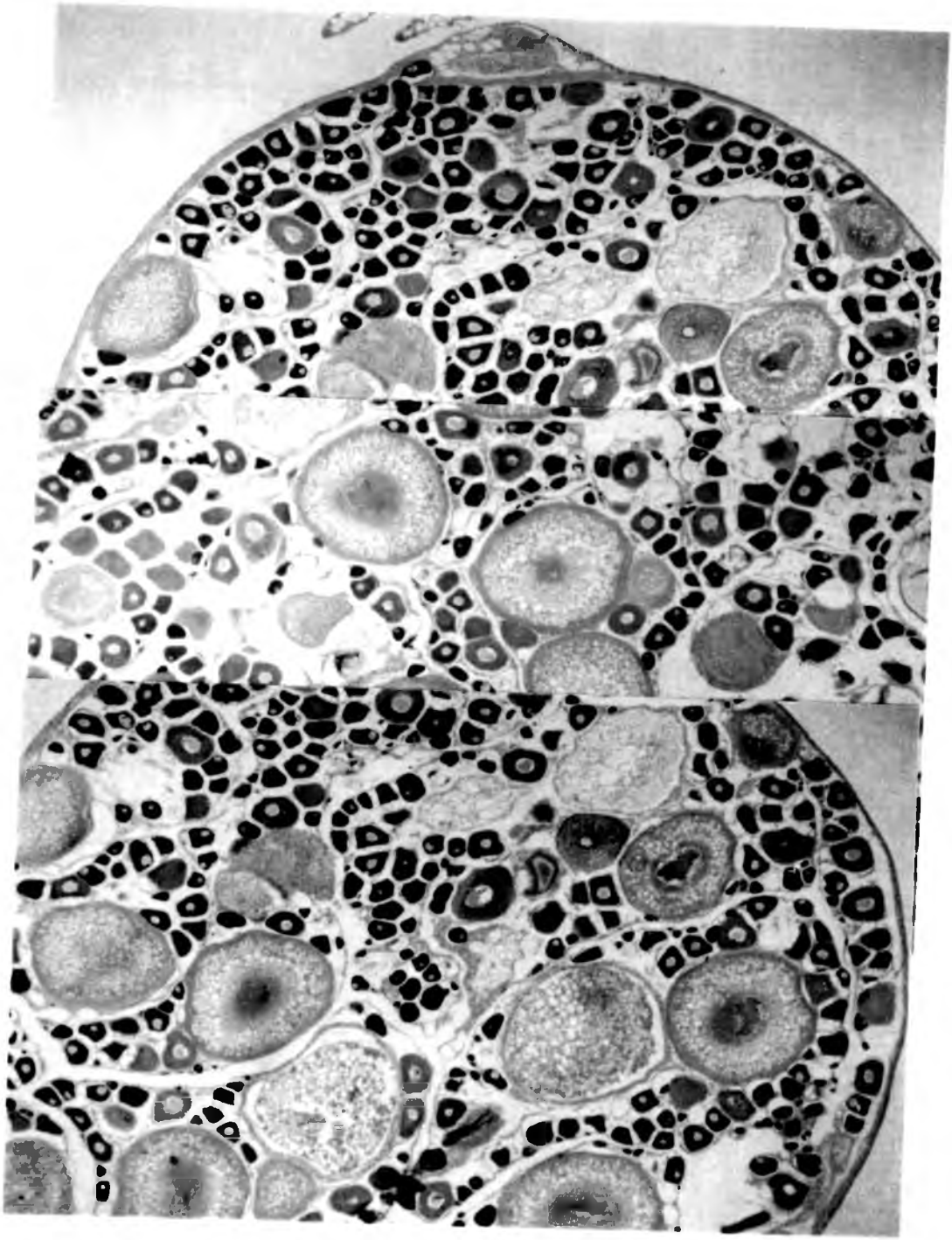


Fig. 4.28 Sections of anterior, middle and posterior part of a previtellogenic ovary (top to bottom). Posterior section showed more mature stages of development. Scale: 1 cm = 0.43 mm

Table 4.1: Data of frequency and size of oocytes when preserved in Gilson's fluid or collected by catheterization. Cortical alveoli are included with the previtellogenic category

Cath. % freq	previt.	vit.	mat.	Gilson % freq	previt.	vit.	mat.
	13	21	66		24	34	42
		16	84		42	11	47
	28.5	54	18		37	58	5
	30	40	30		32	32	36
	41	44	15		51	29	20
	68	32			53	47	
	66	33			99	0.6	0.4
		13	87		24	24	52
		8	92		16	11.5	72.5
	38	62			37	63	
	12.5	87.5			21	79	
	49	46	5		61	34	5
Cath. Size	0.5	0.8	2.3	**Gilson size**	0.3	1.8	2.1
(mm)		0.9	2.3	**(mm)**	0.4	0.85	2.5
	0.5	1.5	2.1		0.45	1.6	1.85
	0.65	1.4	1.9		0.3	1.0	2.0
	0.65	1.5	2.1		0.4	1.25	2.2
	0.5	0.85			0.55	0.8	
		1.2	2.2		0.2	1.2	2.45
		0.9	2.1		0.3	0.7	2.0
	0.4	0.75			0.35	0.7	
	0.5	1.0			0.3	0.75	
	0.6	1.5	1.85		0.5	1.5	1.75

Table 4.2: Two tailed Student's-t test results between catheterization and Gilson's fluid methods

Type of Measurement	Stage	df	t-statistic	$P_{0.05,df11}=2.2$
Frequency	previtellogenic	11	-2.5907	P<0.05
	vitellogenic	11	-0.6977	P>0.05
	mature	11	1.88	P>0.05
Size	previtellogenic	11	0.5620	P>0.05
	vitellogenic	11	0.0826	P>0.05
	mature	11	-0.246	P>0.05

prespawning capacity. Finally, except for natural conditions, individuals under constant conditions showed ovarian desynchronization with respect to their timing of the different developmental stages.

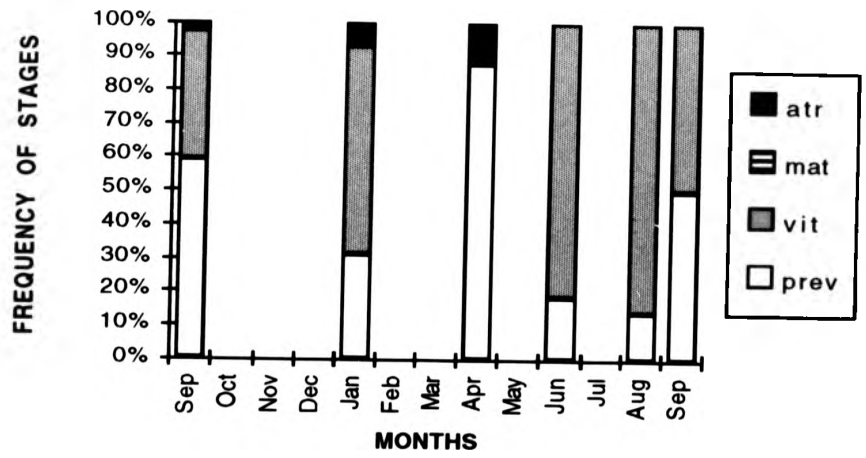


Fig. 4.29a Follow-up of oocytes in individual # A: 7e65 (group A1 then A2), during the second year of constant conditions (22°C; 15L:9D). Advanced spawning occurred during the first year in February.

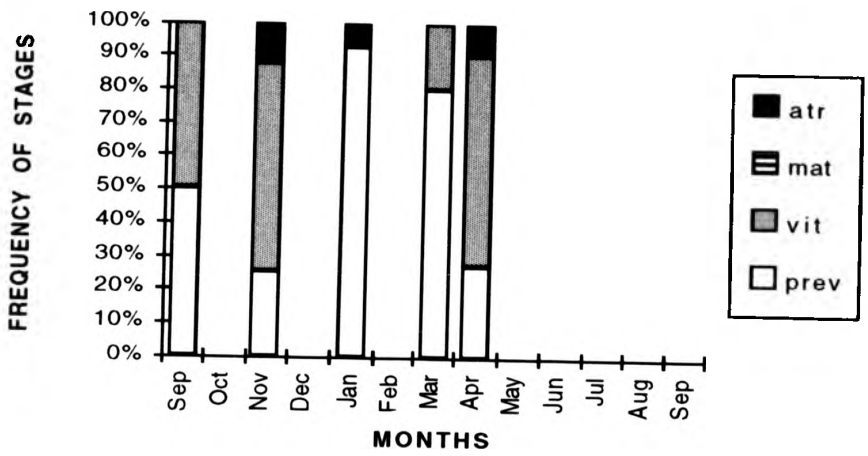


Fig. 4.29b Follow-up of oocytes in individual # A: 7f4e (group A1), during the first yeyear of year of constant conditions (22°C; 15L:9D)

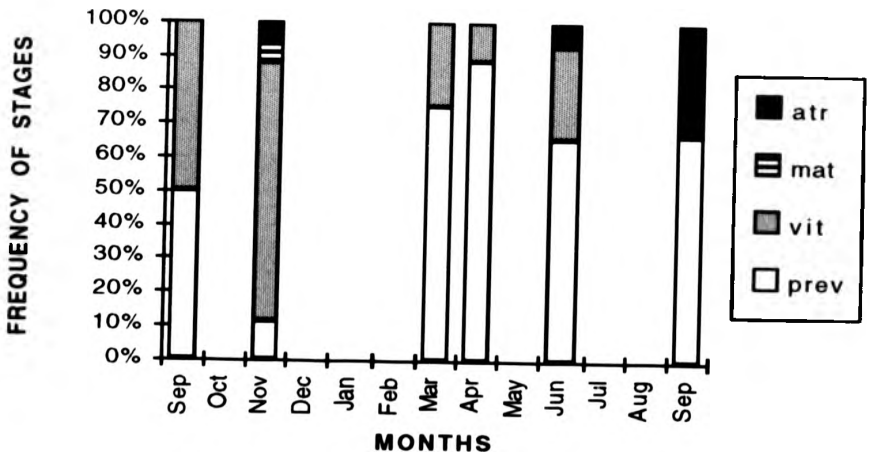


Fig. 4.29c Follow-up of oocytes in individual # A: 208 (group A1 then A2) during the second year of constant conditions (22°C; 15L:9D)

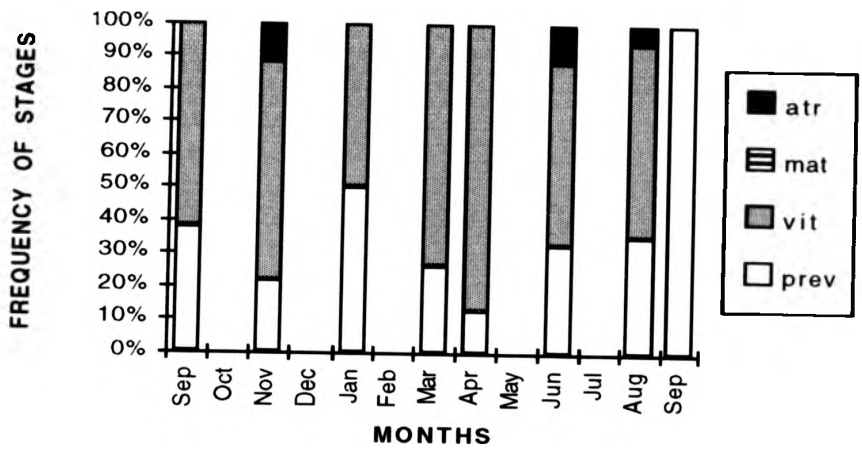


Fig. 4.29d Follow-up of oocytes in individual # A: 228 (group A1 then A2) during the second year of constant conditions (22°C; 15L:9D)

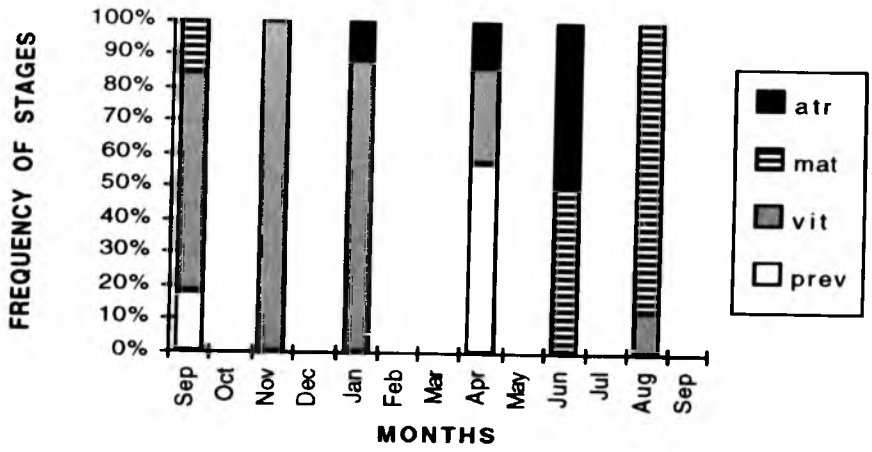


Fig. 4.29e Follow-up of oocytes in individual # D: O435 (group D1 then D2) during the second year of constant conditions (10°C; 9L:15D). Delayed spawning occurred in August.

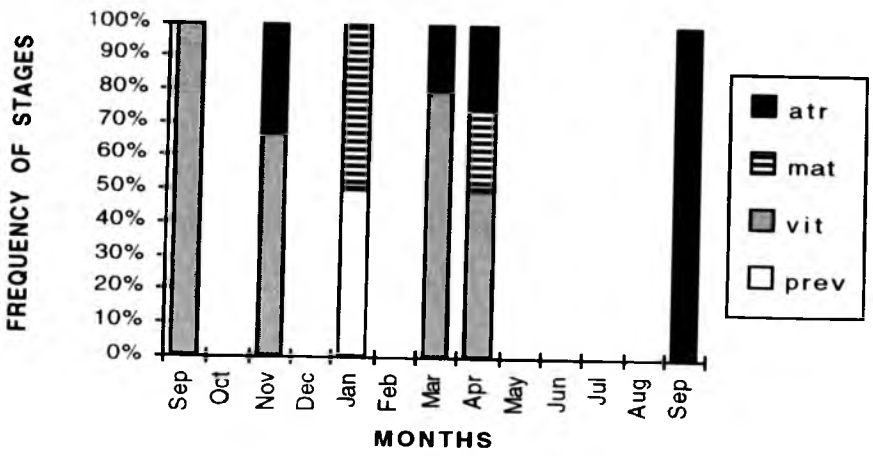


Fig. 4.29f Follow-up of oocytes in individual # D: O707 (group D1 then D2) during the second year of constant conditions (10°C; 9L:15D)

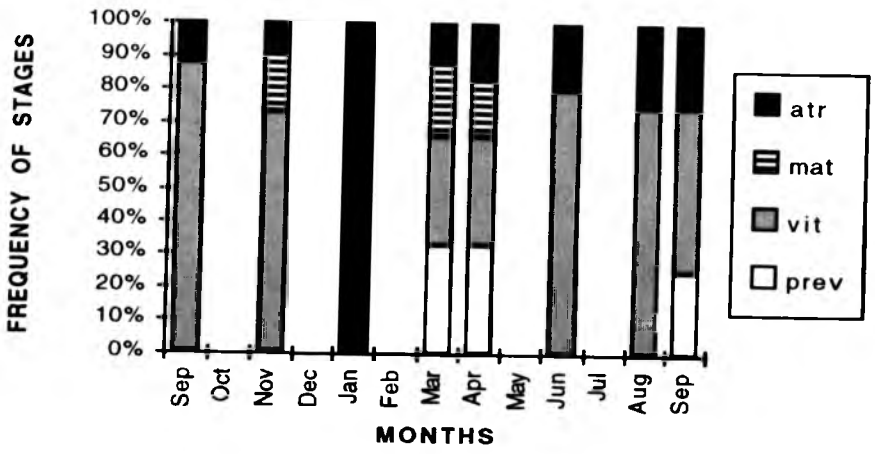


Fig. 4.29g Follow-up of oocytes in individual # D: Oa5D (group D1 then D2) during the second year of constant conditions (10°C; 9L:15D)

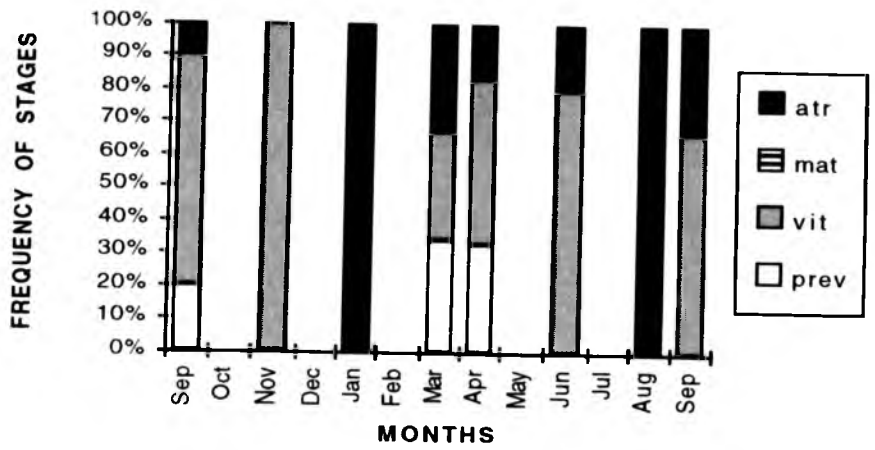


Fig. 4.29h Follow-up of oocytes in individual # D: Oa7B (group D1 then D2) during the second year of constant conditions (10°C; 9L:15D)

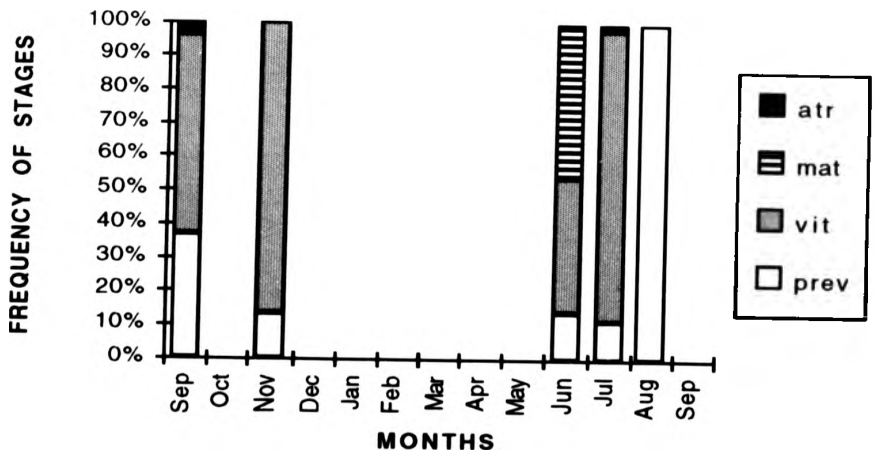


Fig. 4.29i Follow-up of oocytes in individual # N: O10B, control group kept in a pond with the natural seasonal conditions found in Quebec (45°N, 71°W). Natural spawning occurred in May-June.

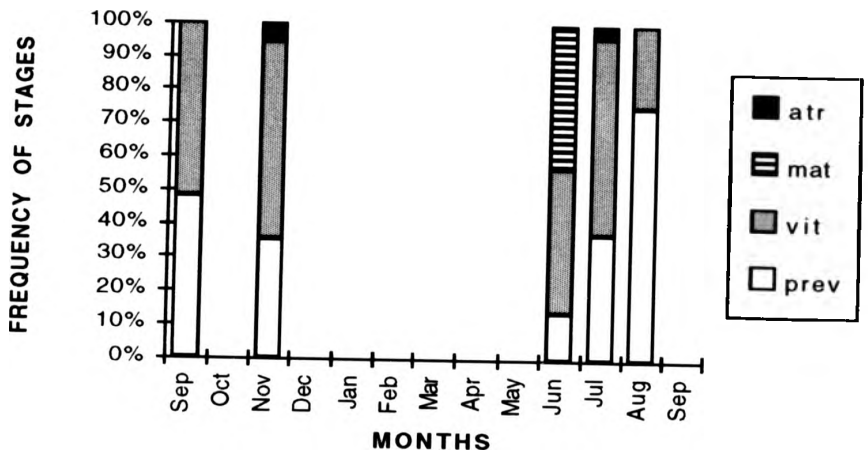


Fig. 4.29j Follow-up of oocytes in individual # N: O1B5, control group kept in a pond with the natural seasonal conditions found in Quebec (45°N, 71°W). Natural spawning occurred in May-June.

During the first year, constant summer conditions led fish to spawn early (January-February) whilst constant winter conditions delayed their spawning by two-three months. Spawning tests were not conducted in the second year.

To illustrate the sequence of changing stages under natural and experimental conditions, data were plotted into bar charts where each bar represented the frequency (% of total occurrences) of a particular stage (Fig 4.30 to 4.34). The ovaries developed more rapidly and attained maturity in January during the first year experiment in summer conditions (Groups A1 & B1) (Fig. 4.30) following a normal seasonal cycle. Fish transferred to the spawning tank spawned between January and March (Chapter 5). The fact that one group (B1) was prevented from spawning during the previous season did not seem to affect the maturation of oocytes. However, during the post-maturation period (May), no vitellogenic oocytes were observed in this non-spawning group. Under winter conditions (Fig. 4.31), group C1 exhibited a high frequency of atresia, particularly during the normal period of spawning (March-May) and no mature

oocytes were observed. Group D1, which had a normal spawning history, showed a slow maturation, the presence of all stages of oocyte development and the constant presence of atresia. In this group, winter conditions maintained a steady state of ripeness which could delay spawning by up to 6 months compared to the normal spawning period (Chapter 5). The second series of experiments with fish in their second year of constant conditions, displayed a different pattern. Under summer conditions (groups A2 & B2) (Fig. 4.32), no mature oocytes were observed. Except for two sampling periods, atresia was always present probably as a resorption process of cortical alveolar and very early vitellogenic oocytes, which could not undertake full maturation. Atretic frequency reached 40%, during September, for the first time spawning group (B2). Previtellogenic and cortical alveolar oocytes accounted, in general, for more than 50% of the total oocytes.

Winter conditions (groups C2 & D2) (Fig. 4.33) showed the presence of mature oocytes and a very high incidence of atresia (up to 70%). The highest frequency of mature oocytes occurred in July, a delay of 2-3 months compared to natural spawning time. In the control group (natural conditions) (Fig. 4.34), perinucleolar and cortical alveoli oocytes were present from June to November. Mature oocytes were present only in May-June during the spawning period. No vitellogenic oocytes were found in August. Gonadal regression at the end of the spawning season has been observed, described and reviewed for a number of species including cyprinids (Hontela & Stacey, 1990), sticklebacks (Baggerman, 1990), seabass (Mayer *et al.*, 1990) and pike (Treasurer, 1990). Atresia for both groups of smallmouth bass was observed only in August probably as a final clean-up of unspawned vitellogenic/mature oocytes. No-preovulatory resorption of oocytes was observed. A month of resorption at the end of summer was also noted. Time to complete resorption varied from 3 weeks in the horse mackerel (Macer, 1974) to 3 months in white sucker (Trippel & Harvey, 1990). Resorption of follicles may be found at any time in the year in goldfish (Beach, 1959), the largest quantities being found at the end of summer when the mature oocytes were reabsorbed and before the start of vitellogenesis. Histological examination of ovaries, at the end of summer, showed atretic oocytes simultaneously with ovarian

recrudescence and the start-up of vitellogenesis. Vitellogenesis started during late August, and by November, early vitellogenic oocytes accounted for 79% of the oocytes. In spring time, clutches of vitellogenic and post-vitellogenic oocytes were maturing until spawning occurred from May to the end of June.

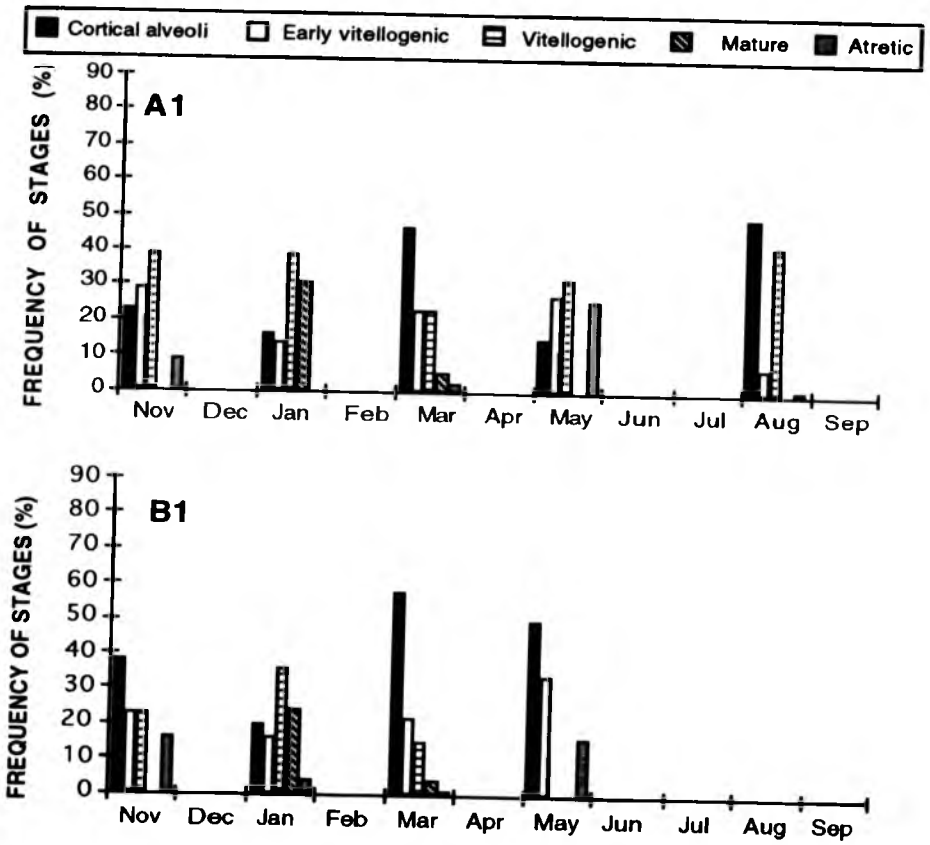


Fig. 4.30 Frequency (% of total occurrences) of oocyte stages from fish kept under constant summer conditions (22°C; 15L:9D) for the first time. Previous to this regime, group A1 had access to normal spawning conditions while group B1 did not. Induced spawning with temperature-photoperiod occurred in March on artificial substrate

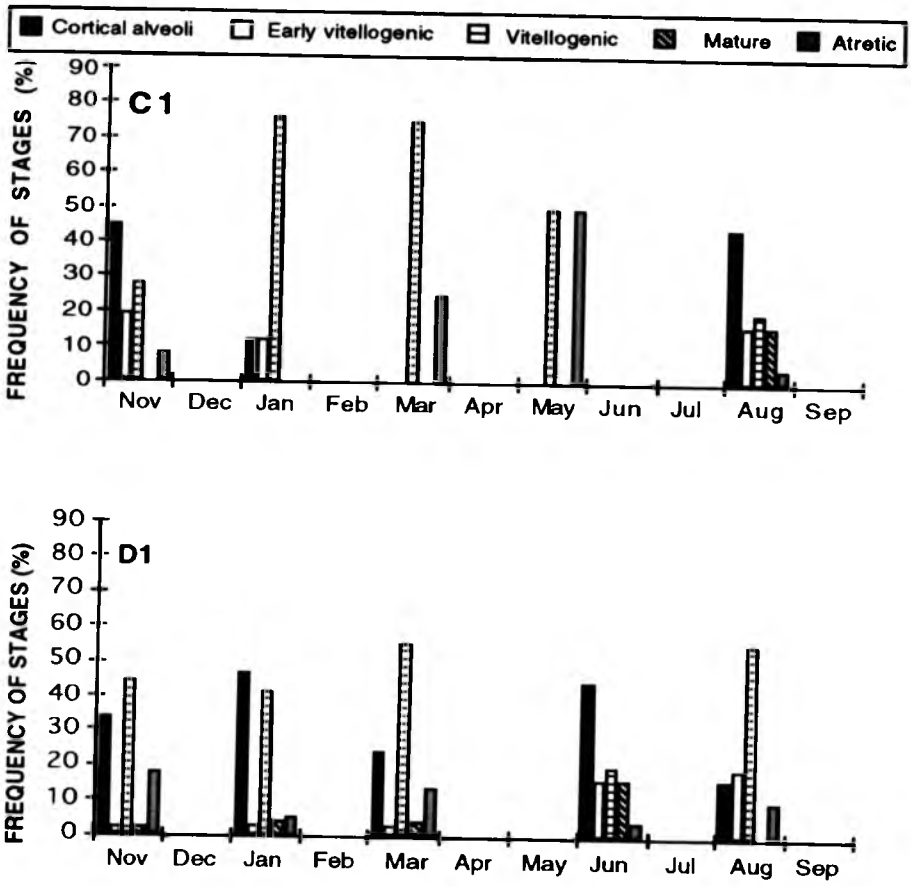


Fig. 4.31 Frequency (% of total occurrences)of oocyte stages from fish kept under constant winter conditions (10°C; 9L:15D) for the first time. Previous to this regime, group C1 had access to normal spawning conditions while group D1 did not. Induced spawning with temperature-photoperiod occurred in June-July- October on artificial substrate

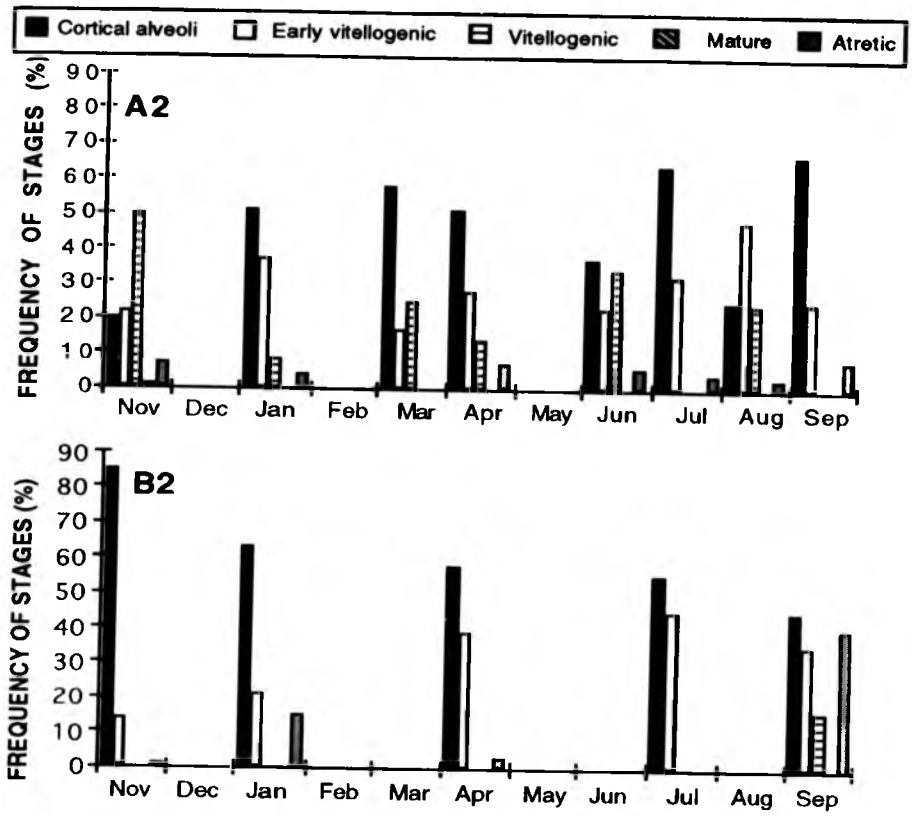


Fig. 4.32 Frequency (% of total occurrences)of oocyte stages from fish kept under constant summer conditions (22°C; 15L:9D) for the second year. Group B 2 was composed of first time spawners while group A 2 had sexually mature fish

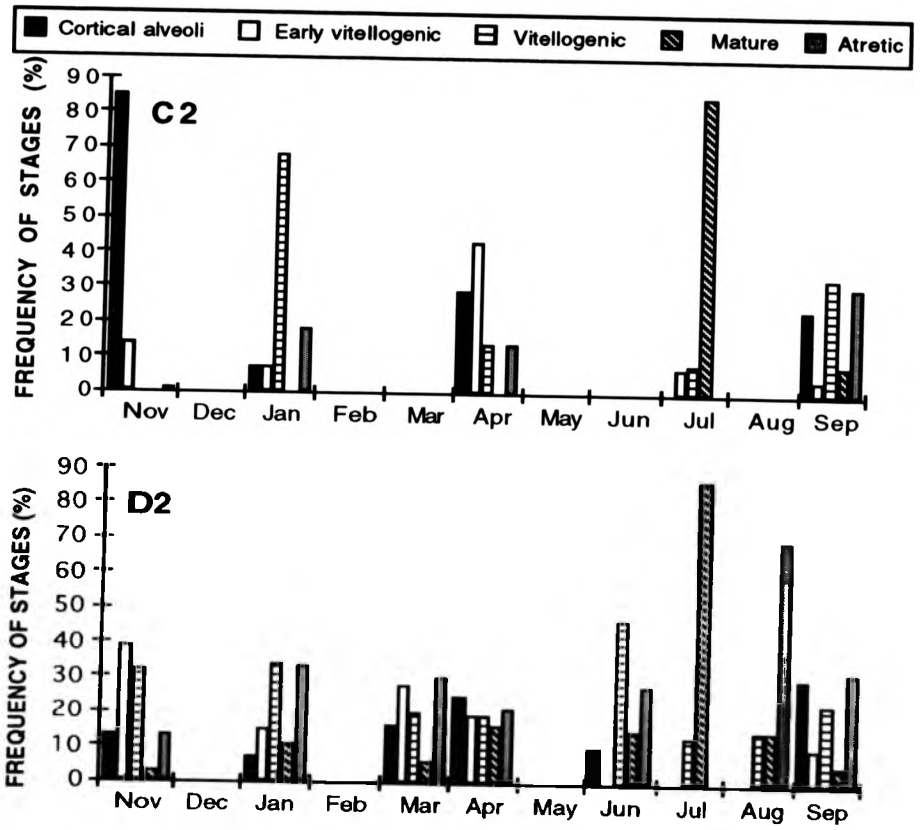


Fig. 4.33 Frequency (% of total occurrences)of oocyte stages from fish kept under constant winter conditions (10°C; 9L:15D) for the second year. Group C2 was composed of first time spawners while group D2 had sexually mature fish

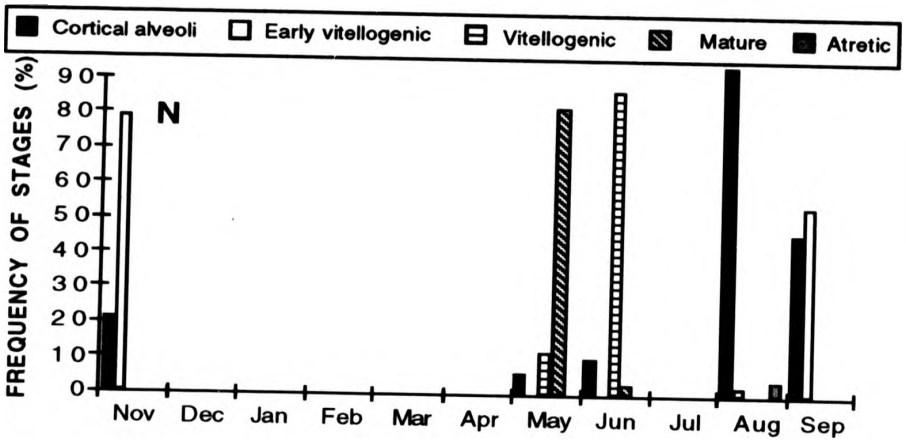


Fig. 4.34 Frequency (% of total occurrences)of oocyte stages from fish kept in a pond (Group N 2) with natural seasonal conditions (45°N, 71°W)

The mean size and coefficient of variation for each stage (Fig. 4.35 to 4.39) and condition were plotted to illustrate the developmental pattern and the influence of environmental regimes (Chap. 6). The diameter of smallmouth bass oocytes from perinucleolar to mature followed seasonal cycles reaching 2.2-2.5 mm at spawning, during the spring time, then decreasing to an average of 0.5 mm following spawning, under natural conditions. The oscillations observed in oocyte diameters may be explained, in part, by the ovarian status of development when the experimentation started and by the direct effect of the photoperiod-temperature regimes. Fish subjected to altered cycles showed oscillations reaching a maximum vitellogenic size during winter indicative of a state of ripeness sufficient to induce out of season spawning. For all groups of spawners and non-spawners placed under constant conditions for the first time, the increase in oocyte size was faster under summer conditions. Winter conditions showed a slow oocyte growth and the presence of mature sizes for as long as six months. The average size of oocytes from any stage was greater in larger (older) individuals and under winter conditions. Variation of size was inversely proportional to the developmental stage reached. In the control group, the size of mature oocytes was smaller than in any other condition, suggesting that when retained within the ovary, mature oocytes can continue their growth before becoming atretic.

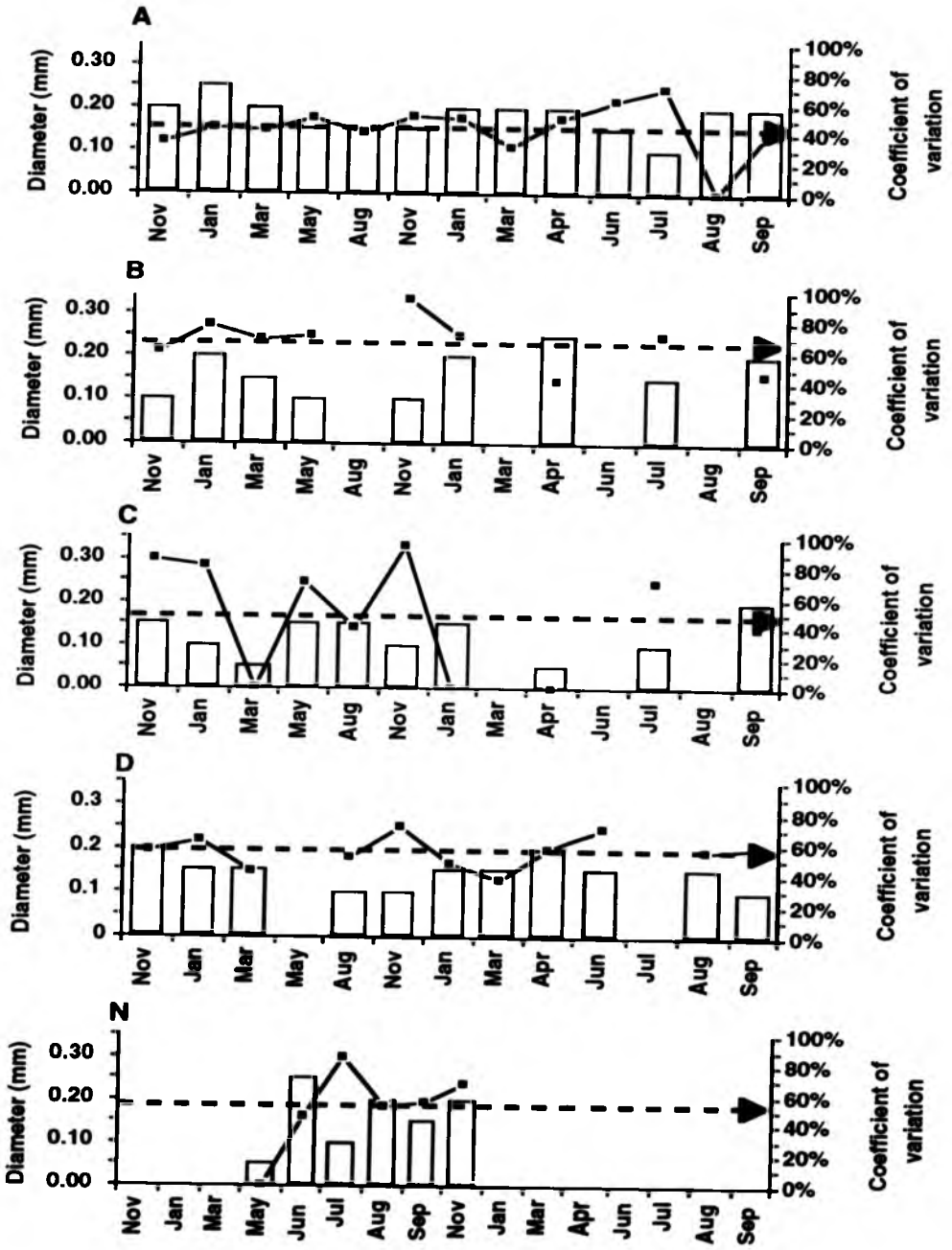


Fig. 4.35. Mean diameter (histogram), coefficient of variation (discontinued line) and mean coefficient of variation (dotted line with arrow)of perinucleolar oocytes in groups A (A1 and A2: spawners; 22°C; 15L:9D), B (B1: prevented from spawning and B2: first time spawners; 22°C; 15L: 9D), C (C1: prevented from spawning and B2: first time spawners; 10°C; 9L:15D), D (D1 and D2: spawners; 10°C; 9L:15D) and N1 (natural seasonal conditions found at 45°N, 71°W)

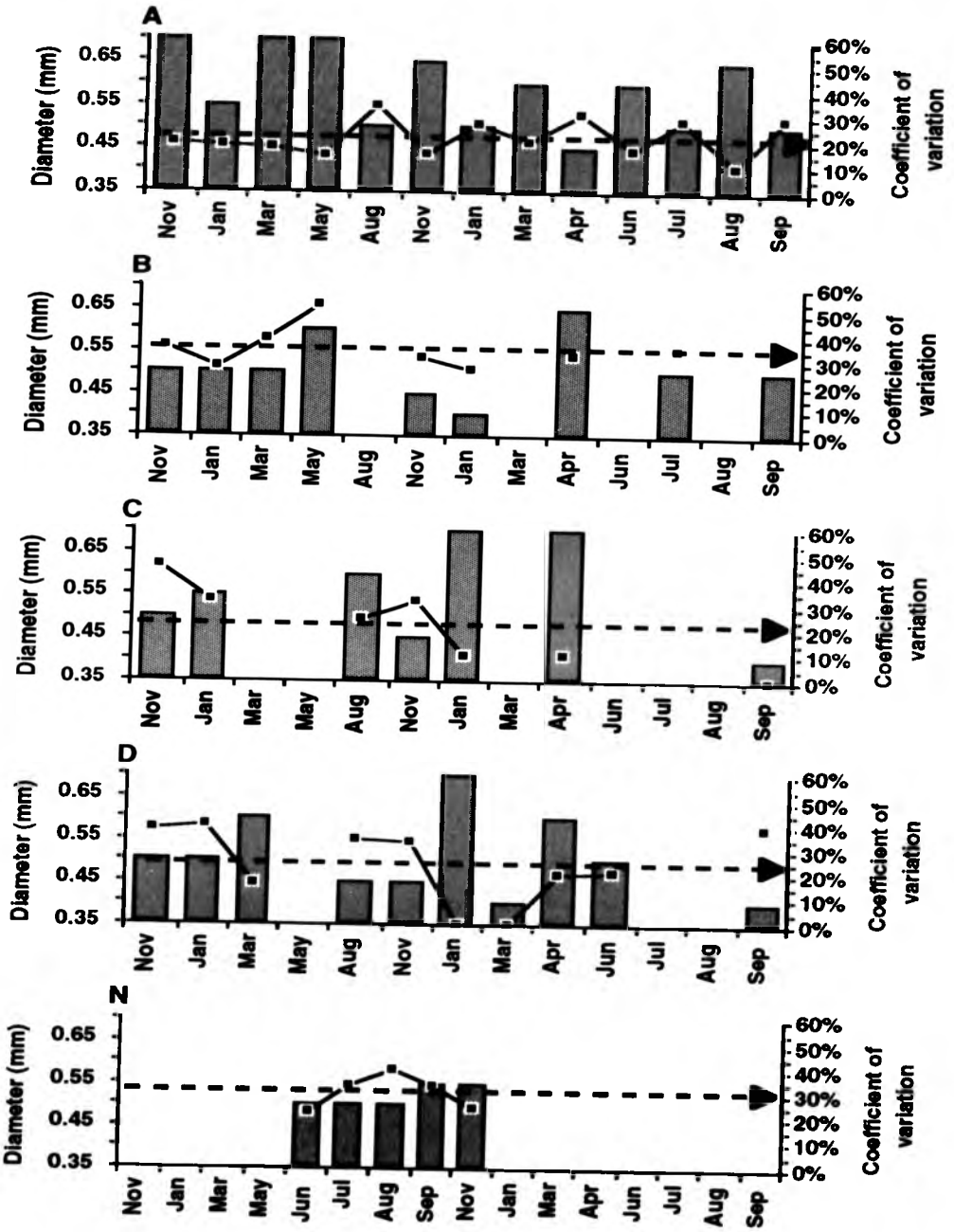


Fig. 4.36 Mean diameter (histogram), coefficient of variation (discontinued line) and mean coefficient of variation (dotted line with arrow)of cortical alveoli oocytes in groups A (A1 and A2: spawners; 22°C; 15L:9D), B (B1: prevented from spawning and B2: first time spawners; 22°C; 15L:9D), C (C1: prevented from spawning and B2: first time spawners; 10°C; 9L:15D), D (D1 and D2: spawners; 10°C; 9L:15D) and N1 (natural seasonal conditions found at 45°N, 71°W)

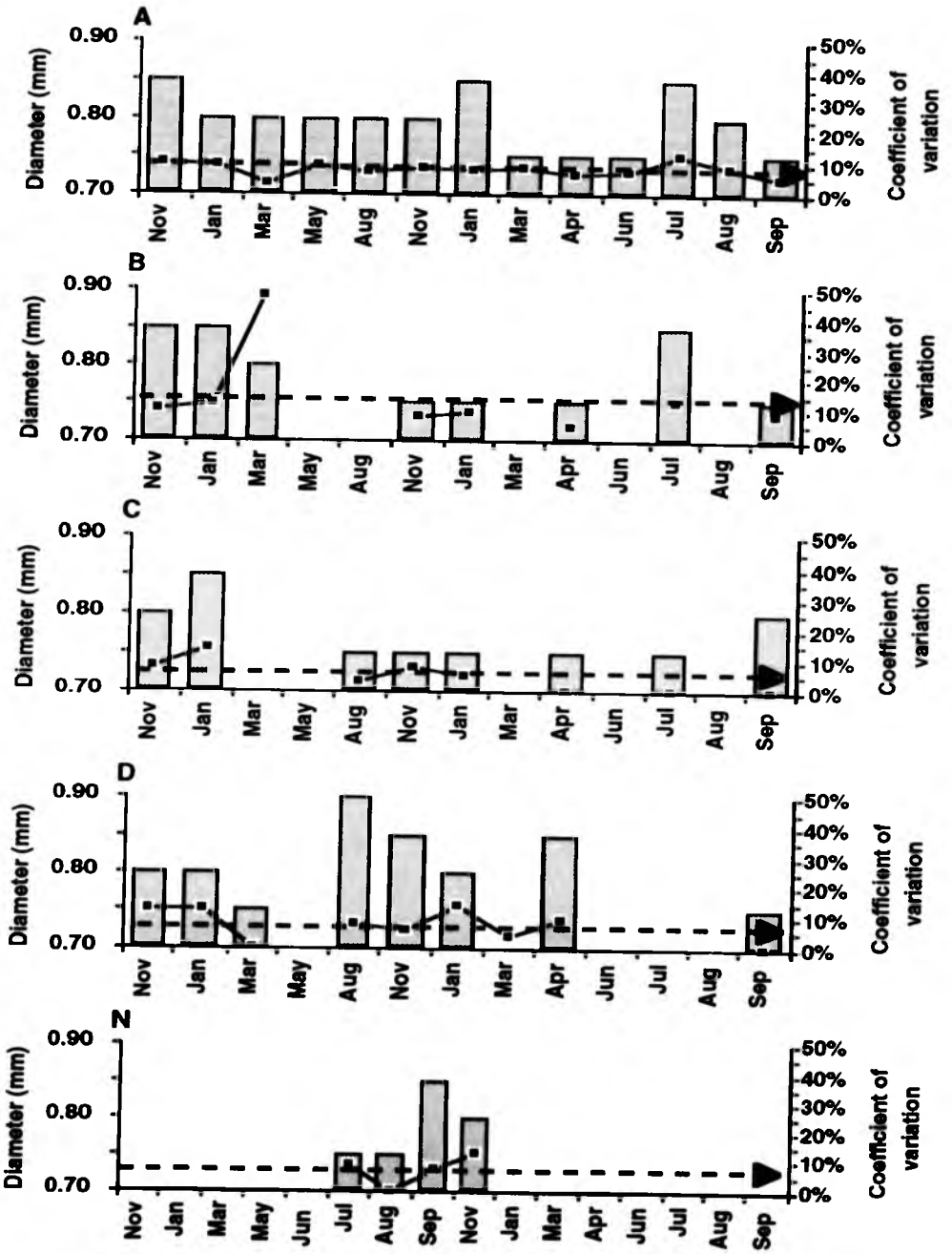


Fig. 4.37 Mean diameter (histogram), coefficient of variation (discontinued line) and mean coefficient of variation (dotted line with arrow)of early vitellogenic oocytes in groups A (A1 and A2: spawners; 22°C; 15L:9D), B (B1: prevented from spawning, B2: first time spawners; 22°C; 15L:9D), C (C1: prevented from spawning. B2: first time spawners; 10°C; 9L:15D), D (D1 and D2: spawners; 10°C; 9L:15D) and N1 (natural seasonal conditions found at 45°N, 71°W)

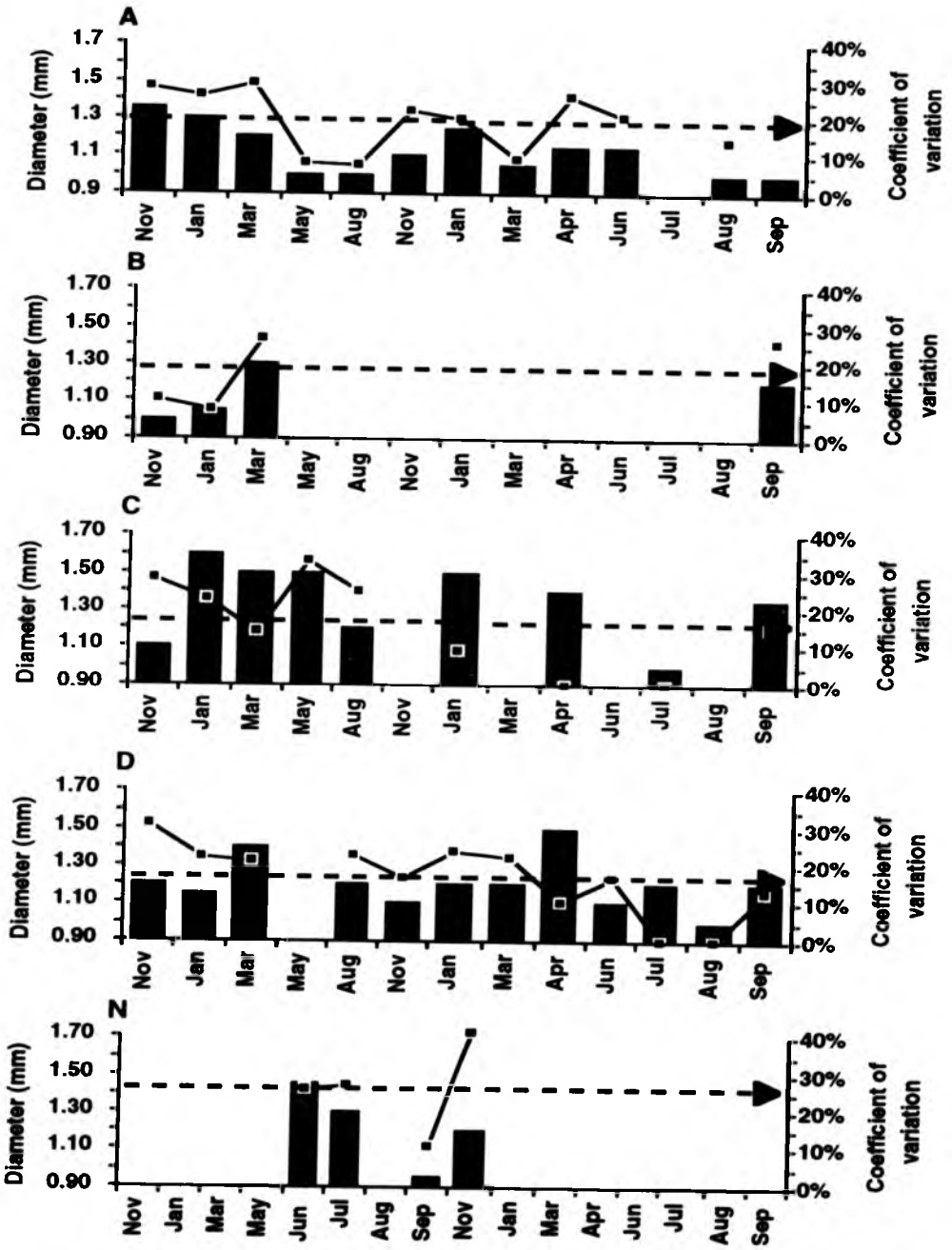


Fig. 4.38 Mean diameter (histogram), coefficient of variation (discontinued line) and mean coefficient of variation (dotted line with arrow)of vitellogenic oocytes in groups A (A1 and A2: spawners; 22°C; 15L:9D), B (B1: prevented from spawning and B2: first time spawners; 22°C; 15L:9D), C (C1: prevented from spawning and B2: first time spawners; 10°C; 9L:15D), D (D1 and D2: spawners; 10°C; 9L:15D) and N1 (natural seasonal conditions found at 45°N, 71°W)

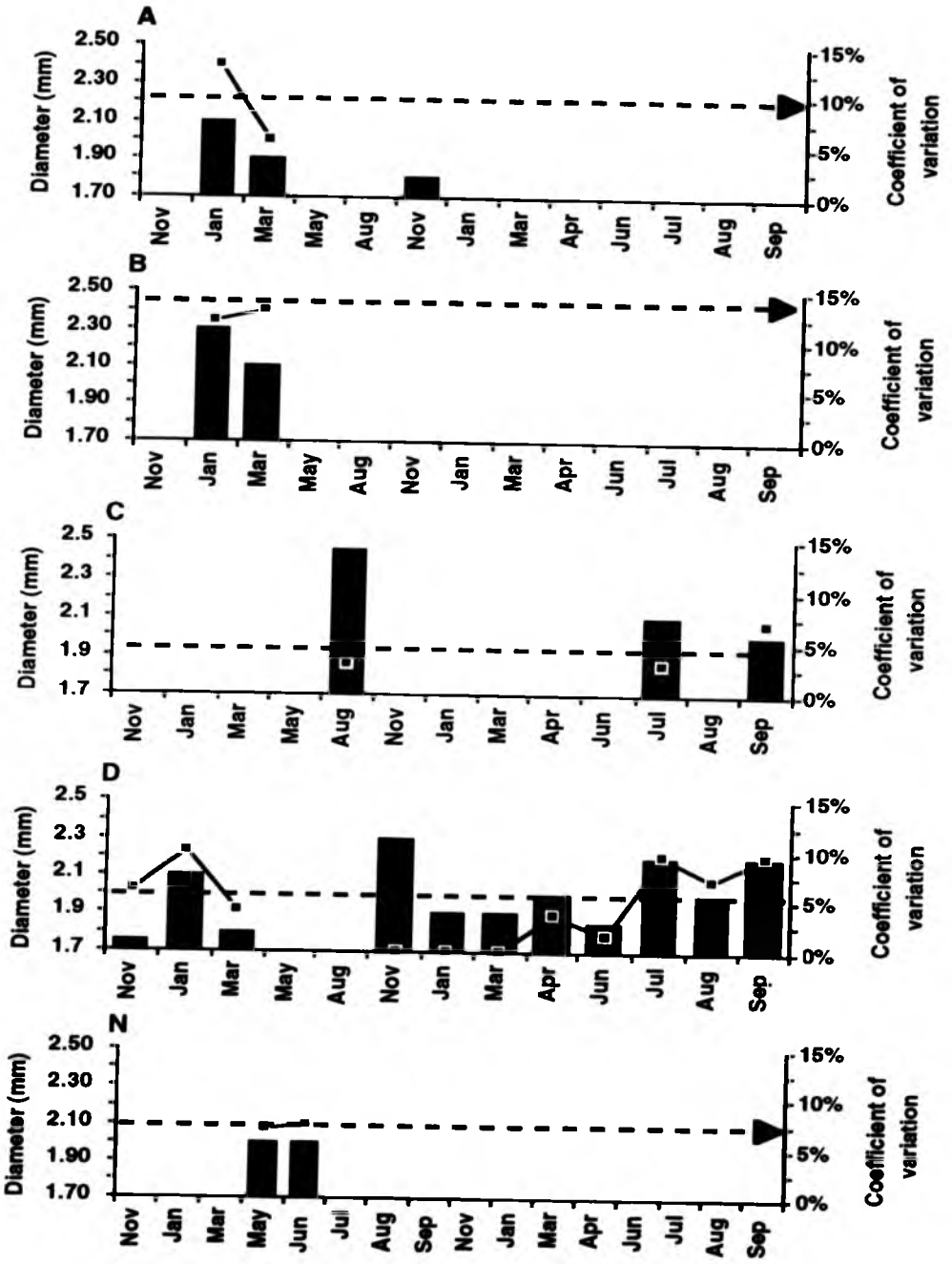


Fig. 4.39 Mean diameter (histogram), coefficient of variation (discontinued line) and mean coefficient of variation (dotted line with arrow)of mature oocytes in groups A (A1 and A2: spawners; 22°C; 15L:9D), B (B1: prevented from spawning and B2: first time spawners; 22°C; 15L:9D), C (C1: prevented from spawning and B2: first time spawners; 10°C; 9L:15D), D (D1 and D2: spawners; 10°C; 9L:15D) and N1 (natural seasonal conditions found at 45°N, 71°W)

4.5 Discussion

The results of the ovarian histology and catheterization have included the sequence of phases already described for other teleosts, e.g. primary growth, secondary growth and vitellogenesis, maturation, ovulation and possibly the formation of a transient *corpus luteum* but have raised many questions. If the oocyte developmental pattern seems similar to other teleosteans, it has shown that there is considerable variability in the development of individual fish. It indicated that the previous photo-thermo-history of the fish can affect the way the fish responds to a stimulus. The pattern also suggested a multispawning capacity through the simultaneous presence of different clutches of oocytes. While it appeared clear that smallmouth bass can maintain mature oocytes over long periods of time anticipating or waiting for the appropriate environmental conditions to occur for spawning, the involvement of the atretic processes is not so explicit. In mammals, the ovarian cycle is characterized by follicular growth, ovulation and the formation of *corpus luteum*. The mammalian *corpus luteum*, of orange-yellow colour in humans, is a transitory secretory gland producing mainly progesterone and lasting from a few days to many months (Eckstein, 1962). In teleosts (reviewed by Xavier, 1987; Jones & Baxter, 1991), the endocrine function of the preovulatory *corpora lutea* remains to be demonstrated while the postovulatory *corpora lutea* of several ovuliparous teleosts is thought to be steroidogenic (Jones & Baxter, 1991).

Our knowledge of ovarian development in teleosts has been based mainly on data collected from domesticated salmonids, particularly rainbow trout and salmon. More recent field information on ovarian development and/or reproductive cycle of fish with small eggs, multispawners and determinate annual fecundity (fixed number of advanced yolked oocytes before the onset of spawning) (Hunter & Macewicz, 1985; Rosenblum *et al.*, 1987; Milton & Blaber, 1991; Hunter *et al.*, 1992) has indicated the incidence and importance of atretic stages more comparable to the results from smallmouth bass. Rosenblum *et al.* (1987) associated atresia in the catfish ovarian cycle with disruption in relation to temperature and low rainfall.

The greatest numbers of early vitellogenic oocytes found under constant conditions were for groups A2 (48%) and B2 (45%) in August and July, respectively. This suggested that a possible role of summer conditions is promoting transformation of previtellogenic oocytes into early vitellogenic stage. Similar results, where vitellogenesis was initiated in the fall, at still elevated temperatures and then continued during winter and accelerated again in the spring, were reported for the channel catfish (Mackenzie *et al.*, 1989). Mature oocytes are normally present in ovaries of smallmouth bass during the spring, and a continual development of the earliest stages maintain batches of eggs ready to be spawned when the temperature reached approximatively 18°C. Under constant winter conditions, the growth of vitellogenic oocytes, the presence of mature oocytes over long periods of time and the continual reabsorption of oocytes, indicated that ovarian development and maturation can cycle for, at least, a year in maintaining fish in a prespawning phase. The presence of different clutches of oocytes was also indicative of a multispawning pattern (Chap. 3).

The concept of individual variability of response has been discussed by Bye (1990) for temperate marine teleosts. It is true that interactions between the environment and reproduction can be complicated by individual variability, and it is certainly enhanced in the case of field investigations and wild animals where captive selection has not yet intervened. As mentioned earlier, the smallmouth bass in this study, were still considered wild. In field studies, it is rarely possible to follow individuals; then if a population spawns over a long period of time, it is not clear if the same individual can maintain the same rhythm or if there is non-synchronization between individuals. The histology itself could not answer this question but the *in vivo* oocyte collection revealed that both aspects were true. An individual smallmouth bass can maintain a steady state of ripening oocytes for an extended period of time, and individuals within a population are not necessarily synchronized. Once conditions conducive to vitellogenic activity are met, transformation of clutches of oocytes of different stages including mature stages may continue for 3-4 months. The frequency of oocytes at different stages displayed a natural pattern in terms of reaching a peak of maturity around natural spawning season followed by atresia, but clutches of vitellogenic oocytes were always

present in fish from any constant conditions. Recruitment of primary oocytes into secondary growth (vitellogenic) was limited by constant winter conditions whilst constant summer conditions limited maturation. This pattern of oocyte development where warm temperatures inhibited further development has also been observed for seabass (Mayer *et al.*, 1990) and cyprinids (Poncin *et al.*, 1987). The sequence of oocyte growth leading to natural spawning can occur independently of seasonal conditions because constant conditions also led to spawning. However, the presence of non-homogeneous clutches of oocytes resulted in variable timing for smallmouth bass reproduction and a delineation of their reproductive rhythm (endogenous). Furthermore, smallmouth bass showed an adaptability to their constant environment by maintaining a continuous state of ripeness or readiness to mature. An endogenous rhythm associated with an acute perception of their environment might partially explain the variability recorded from different studies where smallmouth bass have been reported to spawn anything from one to eight times during the same season (Chapter 3).

The previous cycle as well as the starting point are of importance in studies of reproductive cycle. These factors can determine the speed at which development will occur and modify the balance between the different stages of oocytes. Bromage *et al.* (1984) suggested for rainbow trout that there is a window or period of sensitivity of 6 months to a year in advance of spawning, which can affect the ovarian development. While this might be true for the first clutch of mature oocytes, it seems, from this study, that the capacity of multiple spawning would permit smallmouth bass to readjust the following clutches based on immediate conditions. Natural conditions maintained fish with 2-4 clutches of growing oocytes. First and 2nd year constant summer or winter conditions with fish prevented from spawning maintained, in many individuals, a continuous egg-size distribution. This pattern can be indicative of multiple spawning (Blaxter & Hunter, 1982). The reproductive strategy of smallmouth bass may be modifiable within the same yearly cycle. The experiment started in autumn when all fish showed a high percentage of vitellogenesis. Under constant summer conditions, smallmouth bass matured rapidly and were ready to spawn by January. Under constant

winter conditions, smallmouth bass matured slowly and maintained pre-spawning characteristics until July-August. The second year, with constant conditions, desynchronized the oocyte pattern: continuous egg-size distribution replaced, in many cases, the 2 to 4 homogenous and distinct clutches of oocytes. A lack of synchrony of oocyte development between individuals kept under constant conditions had also been reported in trout (Duston & Bromage, 1986), and a "free running" rhythm was suggested (Chapter 6).

Results from all groups showed that smallmouth bass exhibited group-synchronous oocyte development with two to four clutches of oocytes. While this type is by far the most common strategy amongst teleosts (Wallace & Selman, 1981; de Vlaming, 1983), different recruitment tactics exist. The recruitment may occur directly from oogonia, as in the stickleback (Braeckevelt & McMillan, 1967), or from primary oocytes, as in the herring (Bowers & Holliday, 1961), or from secondary phase oocytes, vitellogenic oocytes, as in *Encrasicholina heteroloba* (Wright, 1992), in the sticklebacks *Gasterosteus aculeatus* and *Apeltes quadracus* (Wallace & Selman, 1979), or from an heterogeneous population of smaller oocytes at various stages of secondary growth, as in the seabass (Mayer *et al.*, 1990). In smallmouth bass, recruitment seems to occur from the cortical alveoli stage, the resting stage at the end of primary growth. Clutches of these cortical alveoli oocytes were present over long periods of time under summer conditions. They were quantitatively dominant at the end of summer (August) in natural conditions, and their numbers were cycling with the appearance of mature oocytes under winter conditions. The maintenance of vitellogenic/post-vitellogenic oocytes, under specific conditions, is not unique to smallmouth bass. The same phenomenon has been reported for multispawners including carps (Davies *et al.*, 1986) and the African catfish (Richter *et al.*, 1987). Histologically, the potential for several spawnings has been related to the presence of synchronously developing secondary growth phase oocytes with simultaneous larger numbers of various sizes of primary growth phase oocytes (Forberg, 1982). This pattern has been observed in smallmouth bass ovaries; some fish displayed clutches of all the stages recorded at one sampling date confirming the multiple spawning potential. The continuous egg-size distribution,

resulting from constant conditions, could also be related to an asynchronous type ovary. Other members of the Centrarchidae family, such as the bluegill, display an asynchronous reproductive cycle (Wiener *et al.*, 1985). However, this reproductive mode in smallmouth bass was limited to out of the ordinary conditions. In the asynchronous type ovary, groups of oogonia are present throughout the year (Srivastava & Singh, 1990). In the present study, oogonia were associated with the presence of atresia and not observed otherwise. Therefore, it would make sense to consider it marginal until there are further studies. Histological examinations of southern populations of smallmouth bass could help clarify the possibility of ovarian asynchrony. The data presented here may also reflect a lack of synchrony in gonad development within a population as suggested for other species (de Vlaming, 1983).

Atresia, in mammals and other groups of vertebrates has been the subject of considerable controversy. In the mammals studied and possibly humans, the atretic oocytes go through quantitative cyclical variations (Ingram, 1962). It means that their number fluctuated with the seasons or with the environmental conditions. In the present study, atresia became very important under the constant winter conditions indicating a high turn-over of oocytes. These particular conditions associated with the atretic processes, maintained a continuous ripeness and the presence of most developmental stages. From the high incidence of atresia associated with the continuous transformation of vitellogenic into mature oocytes, it can be postulated that the mechanism of atresia, either by the endocrinological capabilities of the reabsorbing follicle or/and the direct effect of the oocyte catabolism, is a key in the endogenous synchronization process of the ovarian development. The formation of atretic oocytes is often a response to adverse or inadequate conditions: starvation (Hunter & Macewicz, 1985), overcrowding (Swingle, 1956), sex ratio imbalance (Trippel & Harvey, 1990), and stress associated with environmental conditions (Beamish *et al.*, 1975; McFarlane & Franzin, 1978). It seems logical for the fish to reuse energy by utilizing unfit oocytes into oogonia. But, at the same time, if bad conditions are only temporary, it would be a tremendous energetic loss for the fish to retransform oocytes ready to mature. What if the oviparous teleosts use the *corpora atretica/lutea* to send

information indicating the end of spawning or to prepare for potential spawning? Too little is known about the factors that could influence the onset of atresia and if atresia has one or more different pathways leading to "a wait and see" or "a go situation". Elliott *et al.* (1984) suggested that the testosterone could have a role in the control of atresia and/or yolk resorption. Monitoring of testosterone levels could then become very interesting.

While there are many similarities between species in terms of basic physiology, more evolved species like the Perciformes, particularly fish showing parental care, might rely on a more complex perception of cues influenced by their immediate environment as well as what they can have learned from a previous environment and can anticipate. A relationship between the life of postovulatory *corpora lutea* and the nature of reproductive behaviour in teleosts has been shown (Lam *et al.*, 1978). Fish with simple reproductive behaviour possess short-lived postovulatory *corpora lutea*. On the other hand, the *corpora lutea* last longer in teleosts with complex nesting behaviour (Nicholls & Maple, 1972). It is conceivable that a prolonged preparatory phase (prior to actual courtship) in the behavioral repertoire of the male would increase the probability of a delay between ovulation and oviposition in the female. In this light, an extended life of the postovulatory *corpus luteum* would make physiological sense if it functions in the maintenance of ovulated eggs (Lam *et al.*, 1978). What if the *corpora lutea* also function in the maintenance of non-ovulated oocytes? Further study is needed to assess the role of atresia and possibly consider atretic oocytes more like a *corpus luteum* with some of the endocrine capabilities recognized in other vertebrates. Monitoring the levels of progesterone in fish with high frequency of atresia could possibly provide some link with a steroidogenic capability of the transforming follicles.

SUMMARY

The histological sectioning showed the presence of different clutches of oocytes indicative of a multispawning pattern. Some individuals even displayed a non-homogenous ovary characteristic of asynchronous spawners. Smallmouth bass is a

batch-spawner with the capacity of uni- or multiple spawning in response to environmental conditions. Constant conditions increased the frequency of atretic processes compared to natural/seasonal conditions, and it is suggested that these processes might play a role in the control of spawning.

CHAPTER 5

REPRODUCTIVE RHYTHMS ASSOCIATED WITH GONADO-SOMATIC INDEX, CONDITION FACTORS, AND BLOOD PARAMETERS

5.1 Introduction

Seasonality of reproductive rhythms, in teleosts, has recently attracted research attention, in part, because of the commercial interest in controlling the factors that can modify the natural rhythm and also because of the diversity of fish breeding strategies. Different approaches have been used to define this seasonality including monitoring of morphological and physiological changes *i.e.* changes in the gonado-somatic index (GSI), the condition factors, the development of the oocytes, the plasmatic sexual steroids, proteins and specific electrolytes.

GSI curves can provide information on the reproductive strategies and tactics used by a species to adapt to a fluctuating environment and show the seasonal changes. Changes in condition factor, as discussed in Section 2.6.1, can help identify the direction of change of gonadal development and help follow *in vivo* reproductive seasonal changes particularly when correlated with changes in gonado-somatic index. Variations in total plasma protein levels have been linked to the reproductive annual cycle of rainbow trout (van Bohemen *et al.*, 1981); total plasma calcium and oestradiol-17β (E–2) concentrations have been used as indicators of vitellogenic activity. Protein fractions from gel-electrophoresis of E-2 injected fish served to identify peaks related to high levels of calcium and E-2.

In this study on smallmouth bass, total plasma calcium, total plasma protein, gel electrophoresis and E-2 were measured on spawners as well as on E-2 injected first time spawners to determine values, patterns and correlations with ovarian development.

5.2 Gonado-somatic index

The mean GSI of smallmouth bass, under natural conditions (Fig. 5.1), peaked in May-June followed by a minimum in July with a gradual slight increase during the summer months. Although smallmouth bass spawn during May-June, it is conceivable that fish collected in June had already spawned, at least once, (Chapter 3) and that the absolute GSI value would have been higher than the mean 8.85 recorded. The intraspecific differences of GSI from the fish collected in June (3.27 to 13.9) are an indicator of individual variability and possible mutispawning capabilities (Chapter 3).

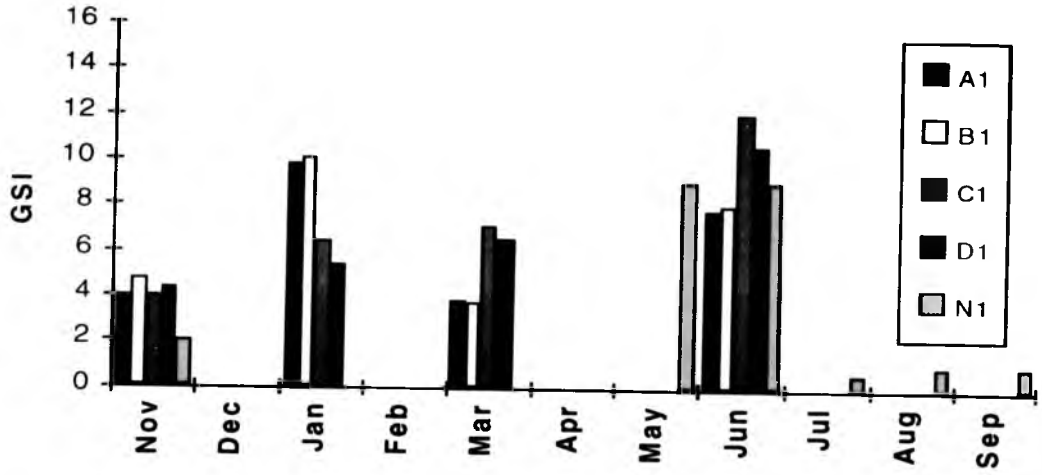


Fig. 5.1 GSI of smallmouth bass during the first year experiment and following seasonal conditions of temperature and photoperiod. Groups C1 & D1: 10°C, 9L:15D; groups A1 & B1: 22°C, 15L:9D; group N1: control in a pond

Simulation of constant summer conditions [Groups A1 & B1: 22°C, 15L:9D], during six months following a seasonal photoperiod-temperature cycle found at our latitude, induced a rapid increase in GSI during December-January with a GSI peak in January and a second peak about 5 months later in May-June (Fig. 5.1). Maintenance of constant winter conditions, over the six months following the same seasonal photoperiod-temperature cycle [Groups C1 & D1: 10°C, 9L:15D] as groups A1 and B1, led to a gradual slow increase in GSI with a peak in May-June (Fig. 5.1).

Two years of constant conditions yielded very different patterns (Fig. 5.2). Summer conditions limited gonadal growth; no peaks were observed. Winter conditions resulted in a normal GSI curve for first time spawners [Group C2: 10°C, 9L:15D] (Fig. 5.2). The winter conditions, however, for previous spawners [Group D2: 10°C, 9L:15D] produced a high GSI from November to April and a peak in July. Although the delay of 2-3 months in the formation of the GSI peak could be expected from fish maintained under winter conditions, the high values observed from November to April indicated a steady state of ripeness (Chapter 4). Clutches of vitellogenic oocytes were also maintained, during winter conditions.

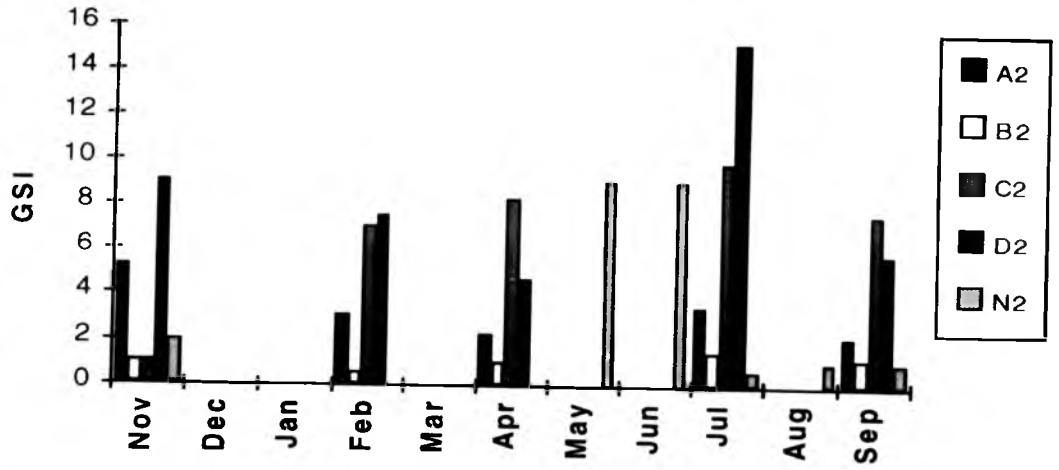


Fig. 5.2 GSI of smallmouth bass during the second year experiment following constant conditions of temperature and photoperiod except for the control group (N2). Groups C2 & D2: 10°C, 9L:15D; groups A2 & B2: 22°C, 15L:9D

The correlation between the GSI and the presence of mature oocytes (Fig. 5.3) was highly significant ($R=0.735$, $P<0.01$); however, only 54% of the variation in the GSI could be explained by the presence of mature oocytes ($R^2=0.54$). The simultaneous occurence of many mature oocytes and a peak in the GSI is to be expected. For the control [Group N: natural conditions] and first time spawners [Group C2: 10°C, 9L:15D], 87% and 85% of the variation in GSI, respectively, was explained by the presence of mature oocytes. Obviously, other factors, such as the photoperiod-

temperature regime and its effect on the growth and development of the ovary, also influenced the GSI.

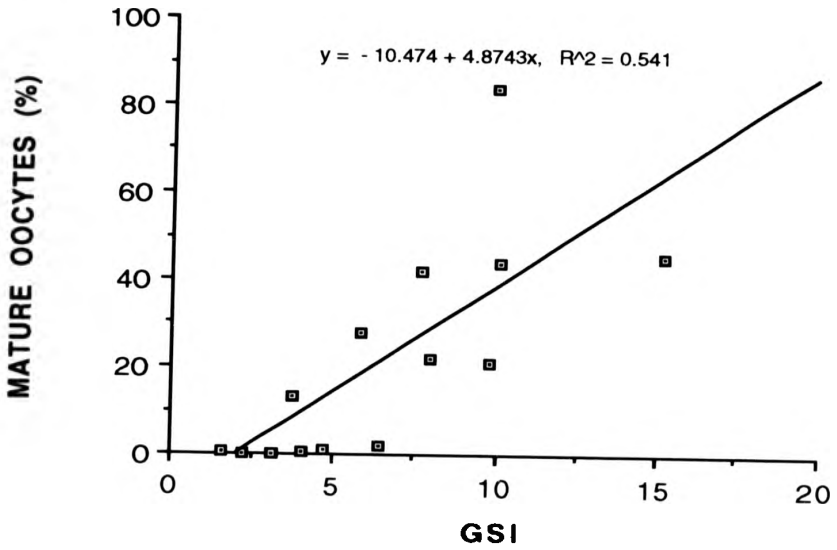


Fig. 5.3 Correlation between the GSI and ovarian development

5.3 Condition factor

The analysis of covariance used to determine the differences between all groups (A1, A2, B1, B2, C1, C2, D1, D2, N, W) demonstrated that the slopes of the length:weight regression were different (Table 5.1). However, comparison of only constant summer conditions, natural spawners and winter spawners (Table 5.2), showed no significant differences ($P>0.05$) between their slopes. Their pooled slope was 3.2 compared with 3.204 obtained from mature smallmouth bass from a PreCambrian lake located in Ontario, Canada (Turner & MacCrimmon, 1970). A Student's-t test demonstrated that there was a highly significant difference ($t_{583}=7.93$, $P<0.01$) between their pooled slope and the number 3, the factor used for isometric growth (Section 2.6.1). When compared with the control and the winter spawners, the groups of bass grown under constant winter condition (Table 5.3) had different slopes. The regression equations of weight on length exhibited by the smallmouth bass kept under winter conditions (groups C1, C2, D1 and D2) indicated a very significant difference ($P<0.01$) when compared with the other conditions. This variability can be explained by behavioural differences

Table 5.1. Covariance analyses for each group and Student's-t test for pooled slope of condition factors;

groups A1, A2, B1&B2: 22°C, 15L:9D; C1, C2, D1&D2: 10°C, 9L:15D; W: winter spawners and Nat.: control group kept in pond

Covariance analyses

Group	Slope	n	df	SSx	SSxy	SSy	df-1	y'		Σx	Σx^2	Σy	Σy^2	Σxy
A 1	3.266	55	54	0.0958	0.3112	1.1316	53	0.121		86.6370	136.5680	159.5130	463.7570	251.5790
A 2	3.0583	152	151	0.1761	0.5369	1.8446	150	0.208		245.1360	395.5160	453.4330	1354.4860	731.8050
B 1	3.1825	61	60	0.6380	2.0356	6.5907	59	0.096		93.1000	142.7300	166.0690	458.7040	255.4950
B 2	3.2239	236	235	0.8727	2.8122	9.2352	234	0.173		349.2160	517.6177	602.8860	1549.3688	894.9200
C 1	2.2243	56	55	0.0755	0.1687	0.5581	54	0.181		88.4870	139.8960	165.4970	489.6520	261.6746
C 2	3.5276	186	185	0.1809	0.6384	2.7347	184	0.481		265.76206	379.9092	457.1189	1126.1633	653.7829
D 1	2.8188	61	60	0.2552	0.7194	2.1784	59	0.151		94.7462	147.4166	174.1573	499.4044	271.2234
D 2	2.5076	166	165	5.3611	10.4286	21.1239	164	0.838		259.0652	409.6669	491.3766	1475.6480	777.2875
W spaw	2.8953	61	60	0.0213	0.0617	0.2815	59	0.103		97.3768	155.4680	181.6304	541.0948	290.0059
Nat spaw	3.2632	24	23	0.2158	0.7042	2.3288	22	0.031		36.8150	56.6886	66.1031	184.3964	102.1037
							1038	2.383	ΣΣ	1616.3413	2481.4770	2917.7845	8142.6746	4489.8770
	within		1048	7.8925	18.4169	48.0076	1047	5.032						
	among		9	4.2469	13.8651	47.9127	9	2.646						
	total	1058	1057	12.1393	32.2820	95.9202	1056	10.073						
			S1	2.3831										
			S2	2.6492										
			S3	2.6459										
			S4	2.3949										
			ΣS	10.0731										
							df	F						
1. Is one regression adequate for all data?							18/1038	186.084						
2. Do lines have same slope?							9/1038	128.210						
pooled slope=within (SSxy/SSx)				=	2.33348									
Student's-t test for pooled slope of condition factors														
3. Is pooled slope diff. than 3?			t=(b-B)/(sqrt(S^2y.x/SSx))											
S^2y.x =	0.005		t (583df)=	-27.00										
SSxy^2/SSx =	42.98		very significant diff P<0.01											

Table 5.2. Covariance analyses for constant summer, control and winter spawners and Student's-t test for pooled slope of condition factors

groups A1, A2, B1 & B2: 22°C, 15L:9D; W: winter spawners and Nat.: control group kept in pond

Covariance analyses

Group	Slope	n	df	SSx	SSxy	SSy	df-1	y'		Σx	Σx^2	Σy	Σy^2	Σxy
A 1	3.2660	55	54	0.0958	0.3112	1.1316	53	0.121		86.6370	136.5680	159.5130	463.7570	251.5790
A 2	3.0583	152	151	0.1761	0.5369	1.8446	150	0.208		245.1360	395.5160	453.4330	1354.4860	731.8050
B 1	3.1825	61	60	0.6380	2.0356	6.5907	59	0.096		93.1000	142.7300	166.0690	458.7040	255.4950
B 2	3.2239	236	235	0.8727	2.8122	9.2352	234	0.173		349.2160	517.6177	602.8860	1549.3688	894.9200
W spaw	2.8953	61	60	0.0213	0.0617	0.2815	59	0.103		97.3768	155.4680	181.6304	541.0948	290.0059
Nat spaw	3.2632	24	23	0.2158	0.7042	2.3288	22	0.031		36.8150	56.6886	66.1031	184.3964	102.1037
							577	0.732	ΣΣ	908.2809	1404.5883	1629.6345	4551.8070	2525.9086
	within		583	2.0198	6.4618	21.4124	582	0.740						
	among		5	1.9332	6.4317	21.5512	5	0.153						
	total	589	588	3.9531	12.8935	42.9636	587	0.909						
			S1	0.7322										
			S2	0.0076										
			S3	0.1535										
			S4	0.0161										
			ΣS	0.9094										
							df	F						
1. Is one regression adequate for all data?							10/577	13.963						
2. Do lines have same slope?							5/577	1.200						
pooled slope=within (SSxy/SSx)				=	3.1992									
Student's-t test for pooled slope of condition factor														
3. Is pooled slope diff. than 3?				t=(b-B)/(sqrt(S^2y.x/SSx))										
S^2y.x =	0.001			t (583df) =	7.93									
SSxy^2/SSx =	20.67			very significant diff P<0.01										

Table 5.3. Covariance analyses for constant winter, control and winter spawners and Student's-t test for pooled slopes of conditions factors

groups C1, C2, D1 & D2: 10°C, 9L:15D; W: winter spawners and Nat.: control group kept in pond

Covariance analyses

Group	Slope	n	df	SSx	SSxy	SSy	df-1	y'		Σx	Σx^2	Σy	Σy^2	Σxy
C 1	2.2243	56	55	0.0755	0.1687	0.5581	54	0.181		88.4870	139.8960	165.4970	489.6520	261.6746
C 2	3.5276	186	185	0.1809	0.6384	2.7347	184	0.481		265.7621	379.9092	457.1189	1126.1633	653.7829
D 1	2.8188	61	60	0.2552	0.7194	2.1784	59	0.151		94.7462	147.4166	174.1573	499.4044	271.2234
D 2	2.5076	166	165	5.3611	10.4286	21.1239	164	0.838		259.0652	409.6669	491.3766	1475.6480	777.2875
W spaw	2.8953	61	60	0.0213	0.0617	0.2815	59	0.103		97.3768	155.4680	181.6304	541.0948	290.0059
Nat spaw	3.2632	24	23	0.2158	0.7042	2.3288	22	0.031		36.8150	56.6886	66.1031	184.3964	102.1037
							542	1.785	ΣΣ	842.2523	1289.0453	1535.8835	4316.3588	2356.0780
	within		548	6.1097	12.7209	29.2054	547	2.719						
	among		5	2.4500	8.3365	29.1425	5	0.777						
	total	554	553	8.5598	21.0575	58.3480	552	6.546						
			S1	1.7846										
			S2	0.9349										
			S3	0.7768										
			S4	3.0495										
			ΣS	6.5457										
							df	F						
1. Is one regression adequate for all data?							10/577	144.598						
2. Do lines have same slope?							5/577	56.785						
pooled slope=within (SSxy/SSx)			=	2.08207										
Student's-t test for pooled slope of condition factors														
3. Is pooled slope diff. than 3?			t=(b-B)/(sqrt(S^2y.x/SSx))											
S^2y.x =	0.005		t (583df)=	-32.15										
SSxy^2/SSx	26.49		very significant diff P<0.01											

(particularly group C1) and by the high rate of atresia observed for these groups. During the first year experiment, group C1 did not feed as well as other groups and always displayed a nervous reaction toward any activity in their vicinity. This can certainly explain, in part, why group C1 displayed a lower slope than group C2 during the following year.

Length and weight of fish from all conditions, without their gonads, were plotted to define smallmouth bass type of growth. The somatic condition factor obtained (Fig. 5.4) had a slope of 3.3044, which was highly significantly different to 3 (t_{43} =2.36, P< 0.01). With this information, individual relative condition factor was calculated based on the slope of its own group. It is interesting to mention that under natural cyclic conditions as well as cyclic conditions with a short winter, there were no significant differences between the slope of their length-weight relation and 3. This indicated an isometric growth under "normal conditions" confirming previous work (Cantin & Bromage, 1991) and an allometric growth in the absence of cyclic conditions.

A second year of constant conditions limited the amplitude of the yearly fluctuations in the relative condition factors (Fig. 5.5) when groups A2, B2, C2, D2 and the winter spawners were compared. These relative condition factors, transformed into a percentage for each group (Fig. 5.6), displayed, in group C2, a curve of ovarian development with one annual peak, in late spring-early summer comparable to the control. Winter spawners had also one peak; however, it occurred in January as a result of a simulated seasonal regime with a short winter and an early simulation of spring. Groups A2, B2 and D2 did not show any peak. In the case of groups A2 and B2, ovarian growth remained in a resting phase (Chapter 4) restrained from further development. Studies on another centrarchid, the crappie (Kaya, 1973) and on some cyprinidae (Poncin, 1989) showed that warm temperature can be the ultimate factor intervening in vitellogenic development. Group D2 showed the highest relative condition factor with a continual slow growth and the least homogeneous range. When compared together, second year constant conditions maintained ovarian growth at the initial level the fish were at at the beginning of the year except for group C2, which displayed the

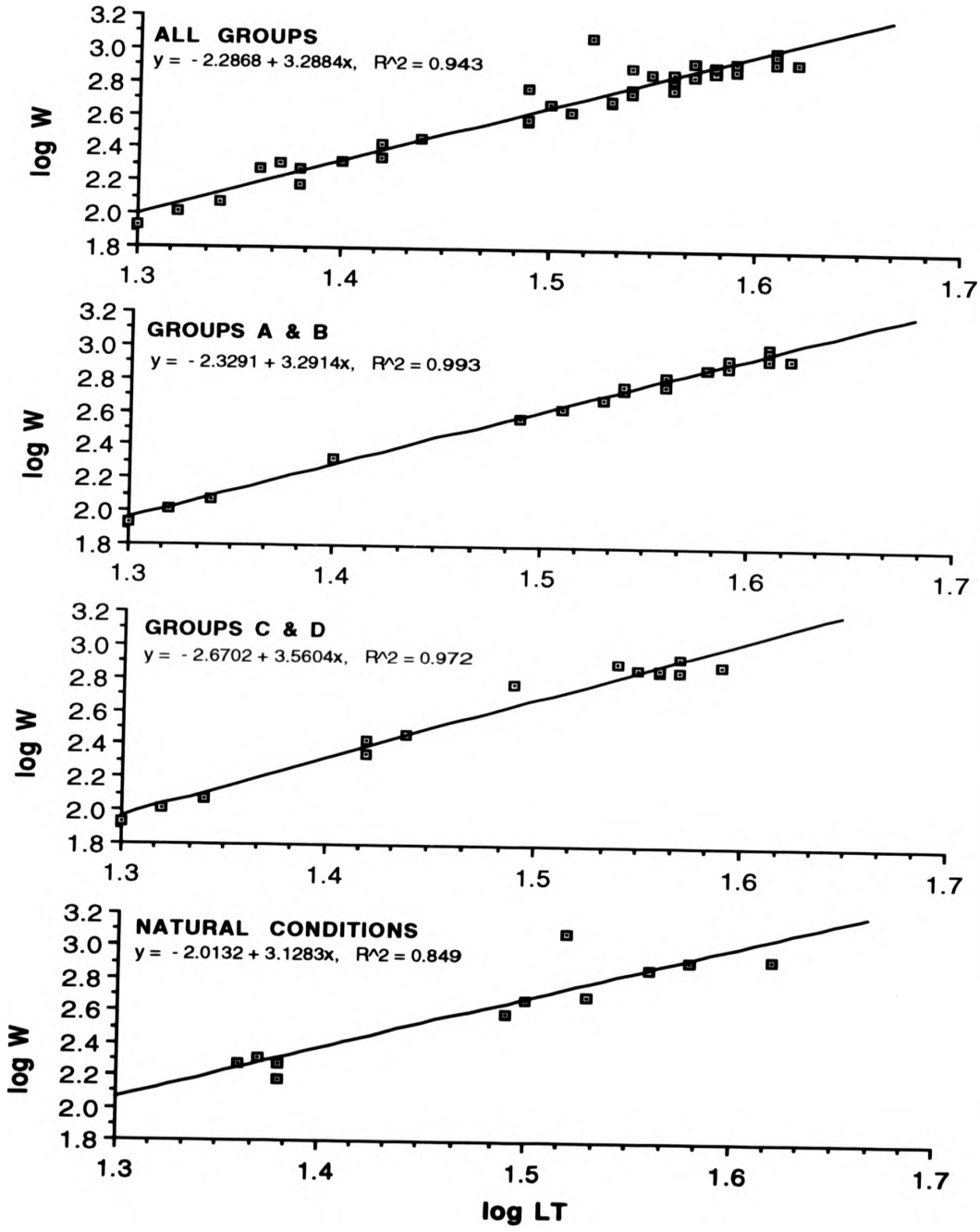


Fig. 5.4 Somatic condition factor (weight of fish - weight of gonads/length^3). Groups C & D: 10°C, 9L:15D; groups A & B: 22°C, 15L:9D and group N: control kept in a pond

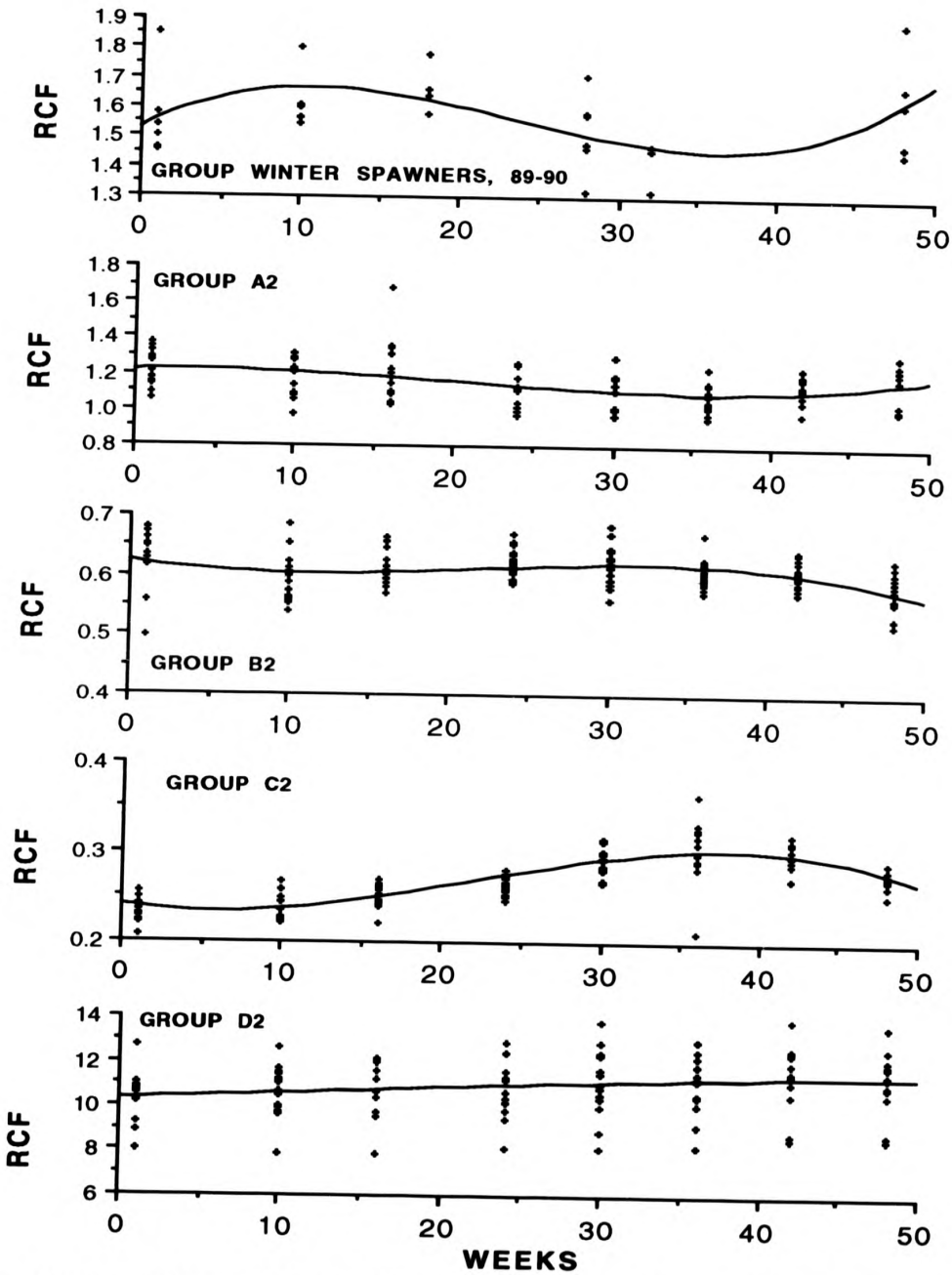


Fig. 5.5 Smallmouth bass relative condition factors (RCF) in 2nd year following constant conditions, Oct-Sept. A2: spawners in 2nd year of constant 22°C- 15L:9D regime, B2: 1st-time spawners in 2nd year of constant conditions, C2: 1st time spawners in 2nd year of constant conditions, D2: spawners in 2nd year of constant 10°C- 9L:15D regime and W: winter spawners

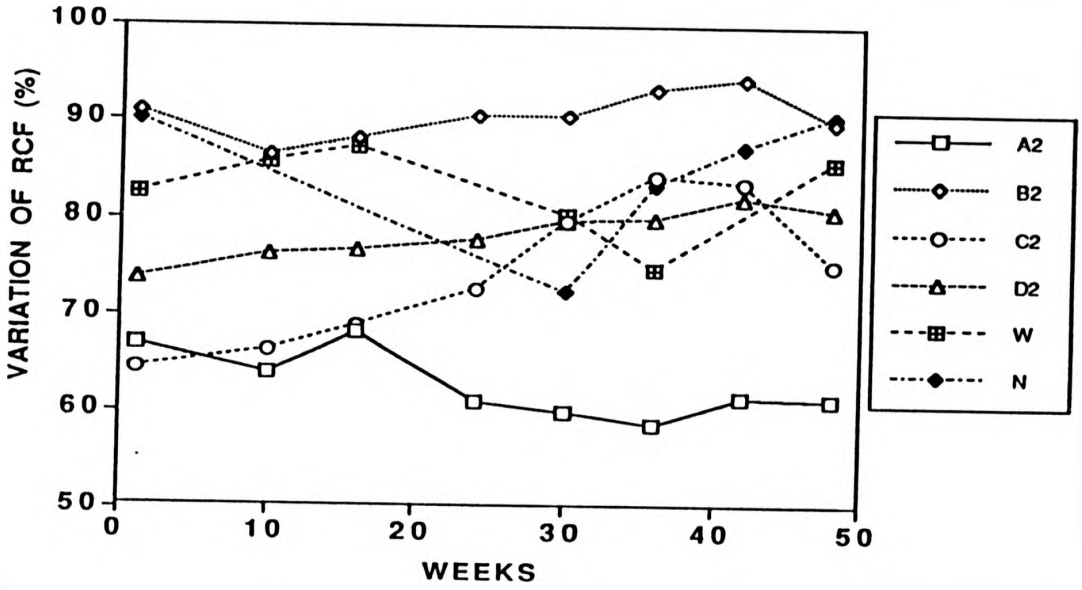


Fig. 5.6 Percent variation of the relative condition factors (RCF) between groups. Groups C2 & D2: 10°C, 9L:15D; groups A2 & B2: 22°C, 15L:9D, group W: winter spawners and group N: control kept in a pond from Oct.-Sept.

expected seasonal variation. Curves C2, W and N displayed a pattern of seasonal oscillation whilst the other curves indicated constant minute oscillations. The relative condition factor has given an overall image of physiological changes; its rhythm has shown desynchronization for most groups except C2 and maintenance of state of ripeness for fish held under constant winter conditions (D2).

The coefficient of variation (C.V.) of relative condition factors (Fig. 5.7) varied between 0.8 and 14% with the highest value for dispersion in groups A2 and D2. This would indicate that a second year of constant conditions could modify the synchronization of ovarian development in mature fish. These results were confirmed by the histological data (Chapter 4) showing "asynchronous" type ovaries with the presence of atretic oocytes.

The correlations between the GSI and the RCF for groups A, B & D (Fig. 5.8) were not statistically significant (P>0.05). The correlation for group C, however, was statistically significant (P<0.05) with 83% of the variation in the GSI being explained by the relative condition factor.

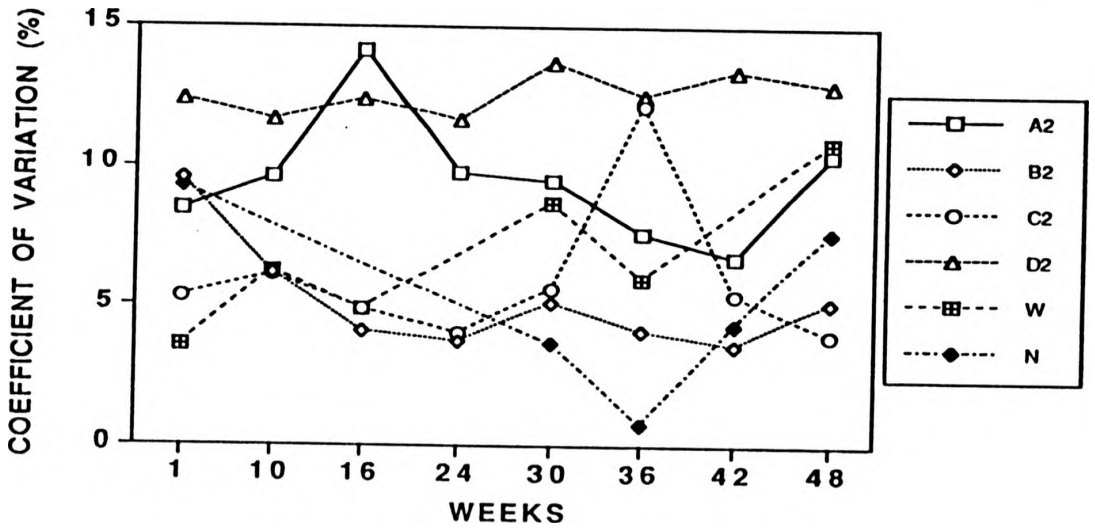


Fig. 5.7 Coefficient of variation of the relative condition factors for all groups. Groups C2 & D2: 10°C, 9L:15D; groups A2 & B2: 22°C, 15L:9D, group W: winter spawners and group N: control kept in a pond from Oct.-Sept.

5.4 Plasma calcium levels

In groups B2, C2 and control (N), total plasma calcium varied between 6.85 and 20.4 mg/dL (Fig. 5.9). The minimum was recorded at the beginning of the experiment (September) for the first-time spawning group and the maximum during the spawning period for the controls. Fish held under summer conditions showed higher values from April on and lower values from October to March. In the controls, total calcium dropped at spawning time from 20.4 to 11.35 mg/dL, stayed low during the summer months then started increasing to reach 14.2 mg/dL in November.

A highly significant correlation (P<0.01) was established between plasma calcium and E-2 (Fig. 5.10). However, only 22% of the variation in concentration of E-2 could be explained by calcium levels.

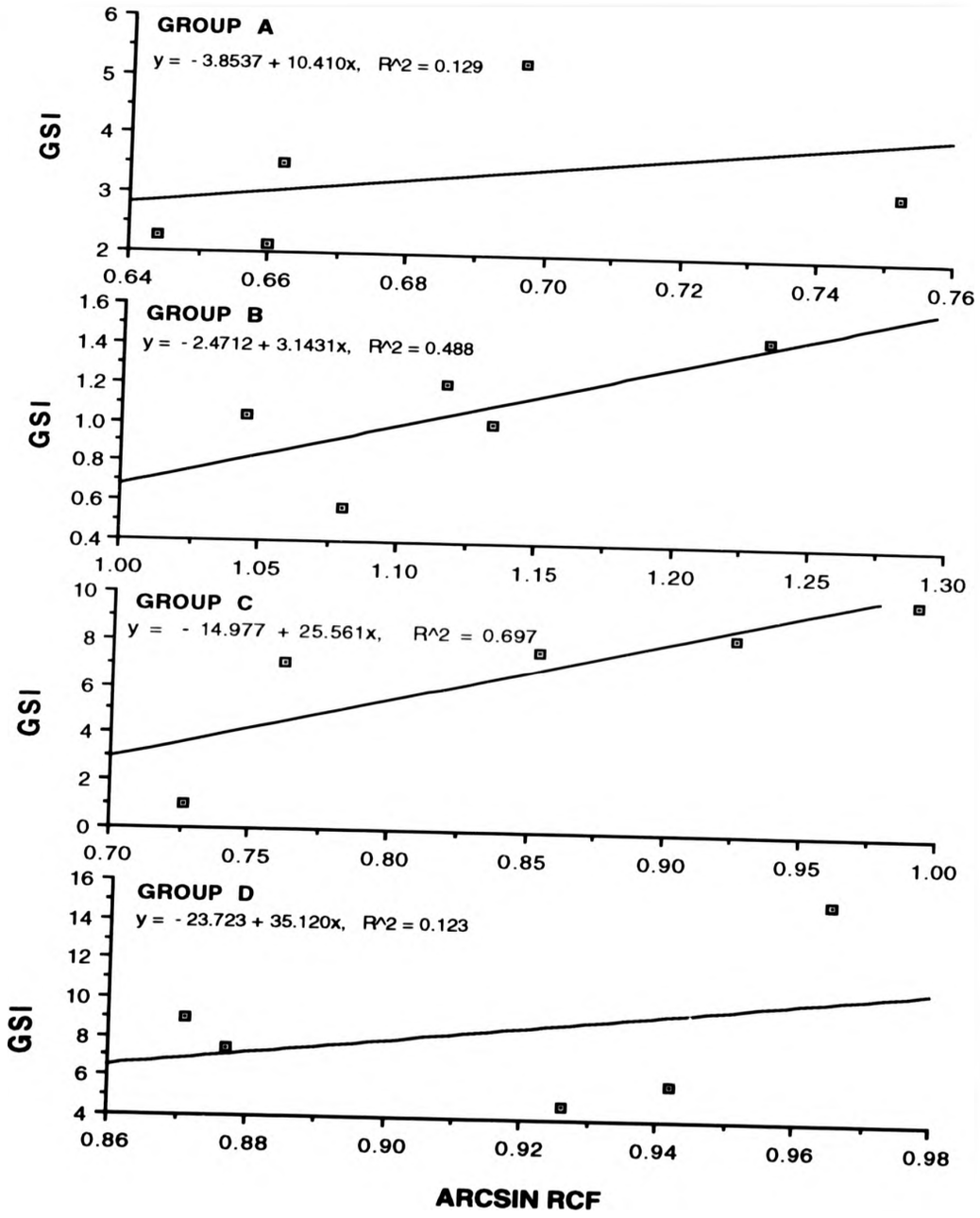


Fig. 5.8 Correlation between the GSI and the RCF of smallmouth bass kept under constant 22°C - 15L:9D photoperiod regime (group A: spawners, and B: non-spawners) and constant 10°C- 9L:15D photoperiod regime (group C: non-spawners, and D: spawners)

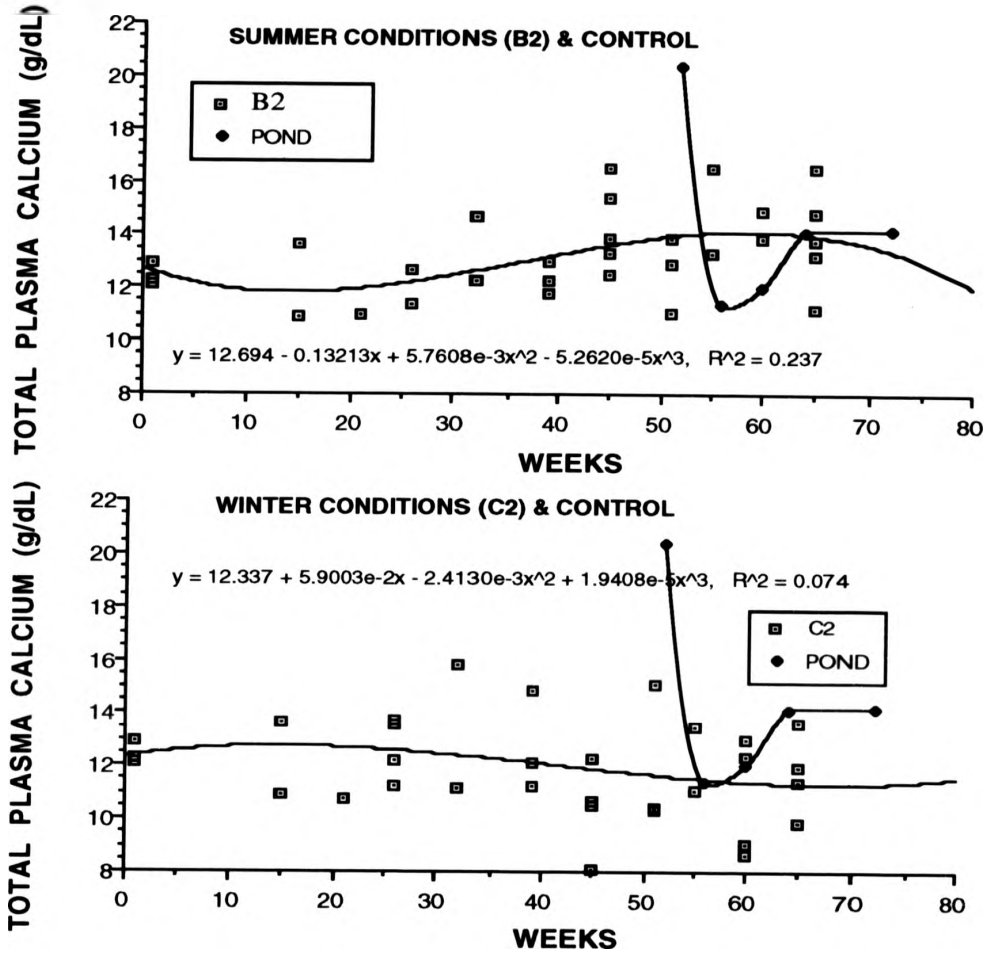


Fig. 5.9 Total plasma calcium in smallmouth bass under natural seasonal regime (control in pond), constant 22°C - 15L:9D photoperiod regime (group B2: first-time spawners) and constant 10°C- 9L:15D photoperiod regime (group C2: first-time spawners) from June -Oct.

A highly significant correlation ($P<0.01$) between plasma calcium and ovarian development was observed (Fig. 5.11); 70% of the variation in the calcium levels was explained by the presence of vitellogenic oocytes. Fish with high numbers of atretic oocytes were not compiled in this correlation, because atresia seemed also to affect calcium levels in a rather erratic way.

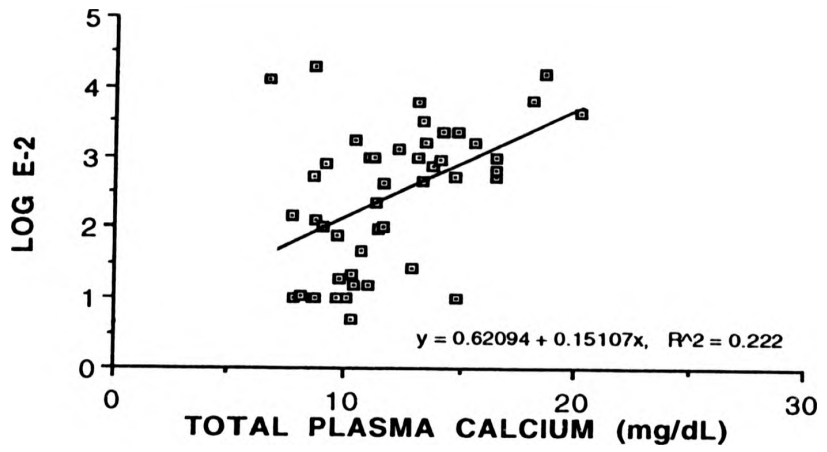


Fig. 5.10 Correlation between oestradiol-17β and total plasma calcium

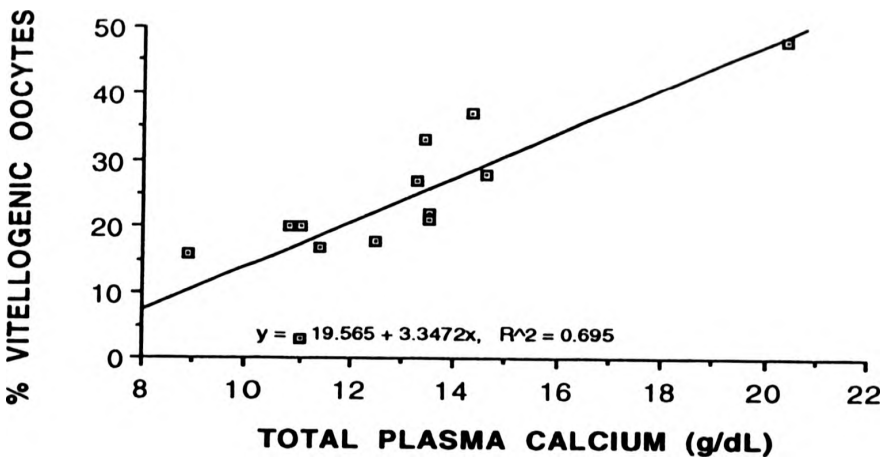


Fig. 5.11 Correlation between total plasma calcium and ovarian development

5.5 Total proteins and electrophoretic profile

5.5.1 Total plasma proteins

In the present study, total proteins varied between 2.3 and 9.8 g/dL (Fig. 5.12); the minimum value was recorded during early ovarian development in first time spawners whilst the maximum value was recorded at the onset of ovarian maturation. The yearly average was almost identical between the constant regimes (4.7 g/dL) and the control (4.9 g/dL). The lowest concentration occured in July-August, in all conditions. Total

protein levels were constantly slightly higher in the plasma of fish kept under winter conditions except for March and August where they dropped lower leading to a similar yearly average. Once again this might reflect the higher level of vitellogenic activity observed for these fish.

A highly significant correlation ($P<0.01$) was established between total protein and plasma calcium (Fig. 5.13). However, only 10% of the variation of total protein was explained by calcium.

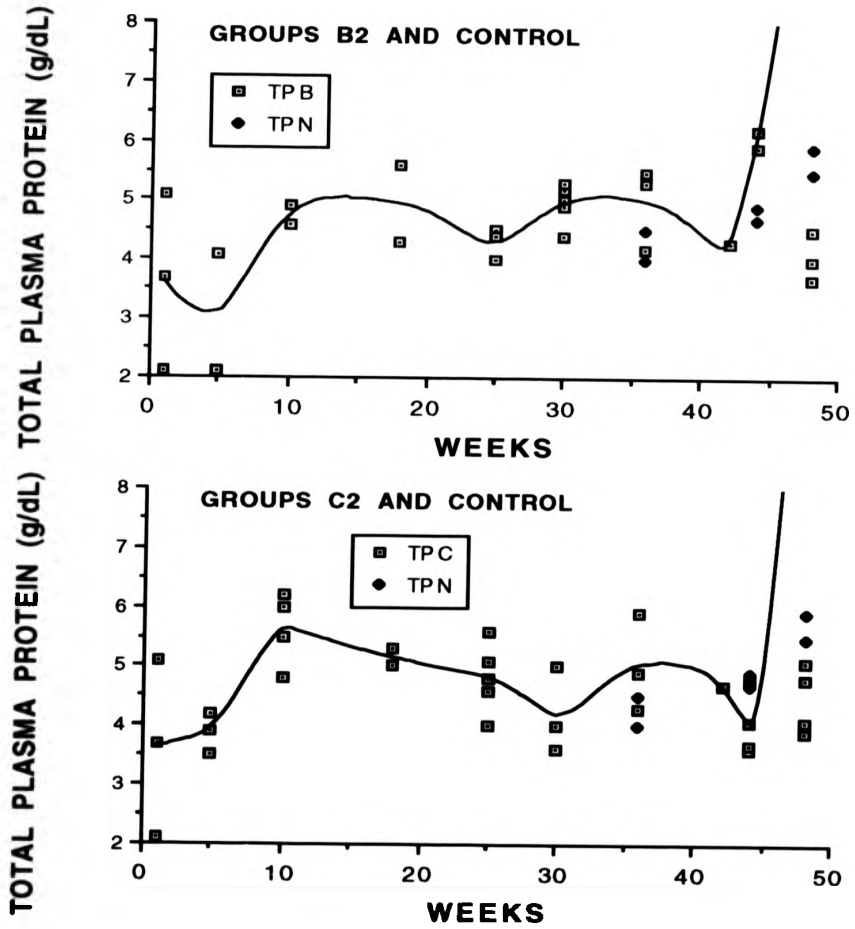


Fig. 5.12 Change in total plasma protein in two groups: constant 22°C - 15L:9D photoperiod regime (group B2: first-time spawners) and constant 10°C- 9L:15D photoperiod regime (group C2: first-time spawners) compared to a control (group N: fish kept in a pond under natural seasonal conditons) from Oct.-Sept.

5.5.2 Electrophoretic profile of proteins

From the five protein components (Chapter 1), only peaks associated with the bands α-1, α-2 and β were evaluated. Individual results (Fig. 5.14) of fish with high concentrations of E-2 have shown a peak located between the α-1 and α-2 bands. Increase in the β band was observed in fish attaining sexual maturity or when induced with E-2. E-2 injections (Section 5.7) resulted in an increase of this peak as well as an increase in plasma calcium. High levels in plasma calcium were associated with a single peak in the β zone.

5.6 Oestradiol-17β (E-2) levels

E-2 levels were measured only during the second year of experiment and on a limited number of samples. Concentrations of E-2 varied between 0.010 and 1.568 ng/ml in groups B2 and C2; high variability was observed amongst individuals for the same sampling period. Levels over 0.2 ng/ml were found in fish with vitellogenic and maturing oocytes held in winter conditions. There was no sampling period where peaks in E-2 concentration were concentrated; constant conditions seem to have enhanced intraspecific differences shown as continuous oscillations between the minimum and maximum, with maxima recorded from April to September. The presence of clutches of vitellogenic oocytes was associated with E-2 concentrations over 200 pg/ml. A highly significant correlation ($P<0.01$) was established between plasma calcium and E-2 (Fig. 5.10).

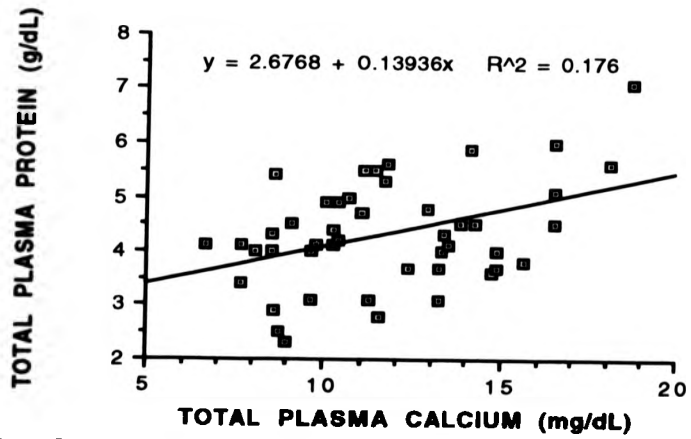


Fig. 5.13 Correlation between total plasma protein and total plasma calcium

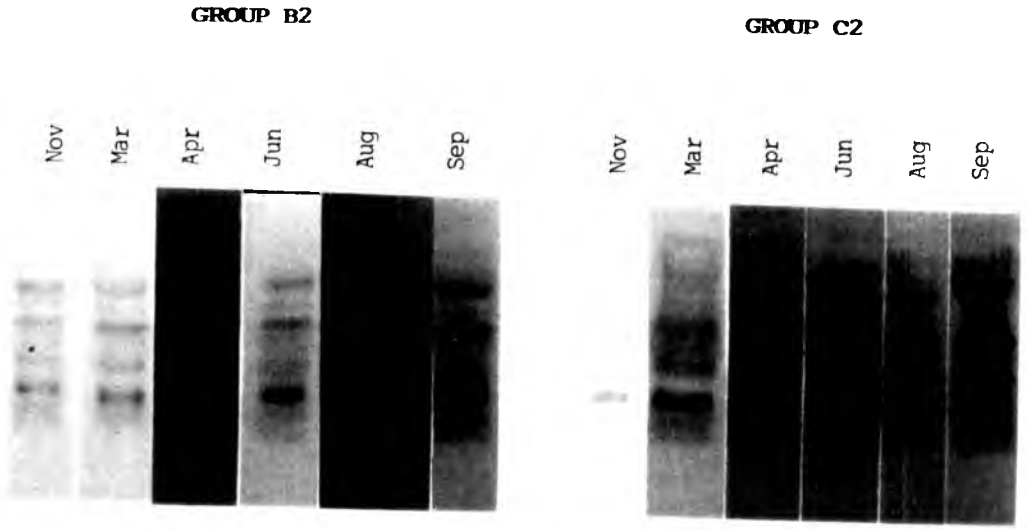


Fig. 5.14 Individual electrophoretic profiles of plasma from fish of group B2 (constant 22°C - 15L:9D photoperiod regime) and C2 (constant 10°C- 9L:15D photoperiod regime)

5.7 Effects of E-2 injection

Injections of E-2 increased oestradiol titre, plasma calcium and total proteins in all groups treated independently of the temperature (Fig. 5.15). E-2 peaked one week post-injection and then decreased but less rapidly under winter conditions. During the same period, a continuous but rather slow decrease was observed in the controls. Plasma calcium responded after more than a week post-injection by a rapid increase in fish under winter conditions (C2). Total protein increased slowly, in both conditions, whilst again the controls followed the same pattern as calcium. The concentration of plasma calcium and total protein increased in the control group B2 whilst they decreased in the control group C2.

Injections of E-2 increased the plasma levels of E-2 up to 19.893 ng/ml, one week post-injection; three weeks later, levels were down to 0.85 ng/ml. Subsequent to the injection of E-2, plasma calcium and protein levels were augmented; however, while calcium

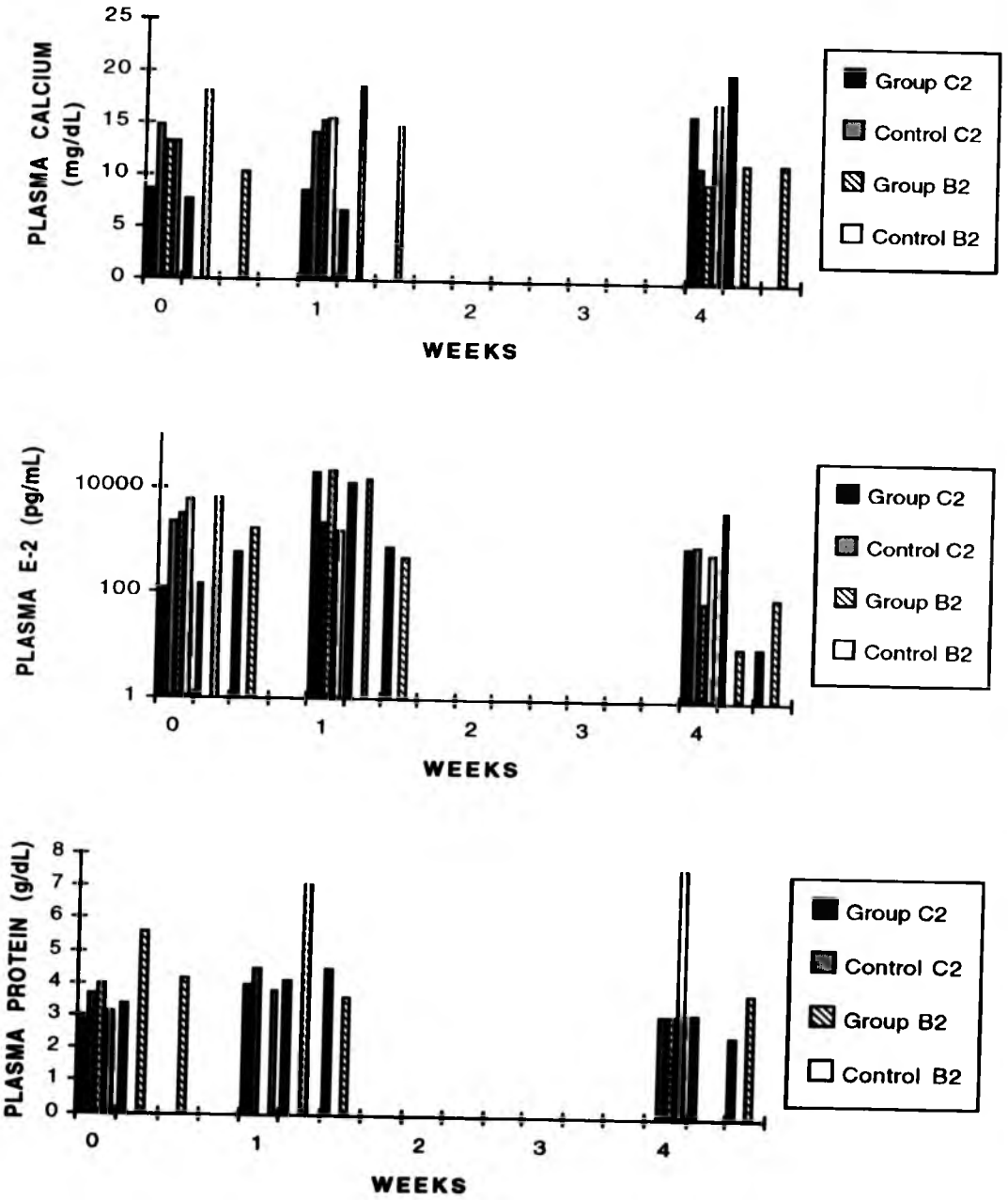


Fig. 5.15 Effects of injection of oestradiol-17β on levels of total plasma calcium, E-2 and total plasma protein in groups B2: 22°C, 15L:9D and C2: 10°C, 9L:15D from Oct.-Sept. The controls were fish injected with vehicle only.

levels increased slowly under winter conditions, proteins levels were higher one week post-injection then decreased by the fourth week independently of conditions. It is worth mentioning that plasma calcium at time 0 was, in some cases, already high and could have partially hidden the increase.

The controls indicated, over the experimental period, an increase in total protein under constant 22°C with 15L:9D photoperiod and a decrease under constant 10°C with 9L:15D photoperiod. The same pattern was observed for plasma calcium.

The electrophoretic profiles (Fig. 5.16) showed an increase of the β fraction subsequent to E-2 injection. Under summer conditions, this increase was registered one-week post-injection whilst under winter conditions, it was recorded after 4 weeks.

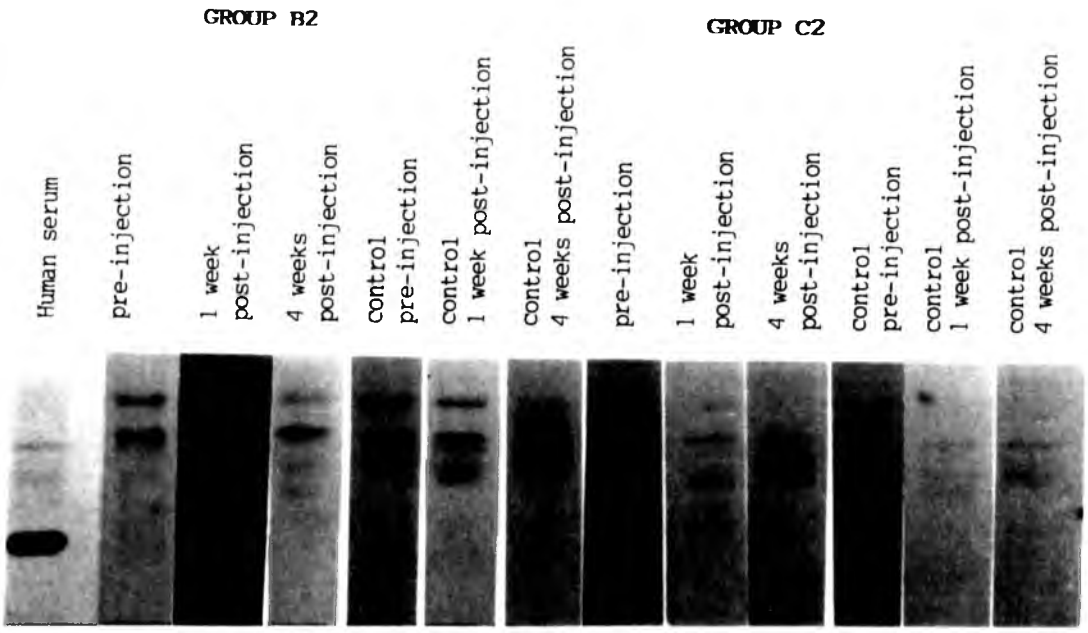


Fig. 5.16 Electrophoretic profiles from individuals in group B2 (constant 22°C - 15L:9D photoperiod regime) and C2 (constant 10°C- 9L:15D photoperiod regime) following the E-2 injection

5.8 Discussion

GSI, RCF and plasma concentrations of oestrogens directly or indirectly measured all varied with the ovarian cycle. Natural patterns derived from the data of the control (group N) and the winter spawners (group W) indicated that GSI and RCF peaked with oocyte maturation, and total plasma calcium, total proteins and E-2 increased with vitellogenic activity. Results from constant photoperiod-temperature conditions in groups A, B, C and D showed continuous oscillations particularly evident for the RCF curves. Bromage *et al.* (1982b) found that spring spawning rainbow trout generally show a peak of oestradiol-17β 2-3 months before spawning and a peak of serum calcium at spawning. Relating this to ovarian development, Lessman & Habibi (1987) reported a peak of oestradiol well before oocyte growth stops, indicating that decreasing levels of plasma oestradiol are associated with final growth and maturation of oocytes. Several studies on species with single annual spawnings (Billard *et al.*, 1978; Kagawa *et al.*, 1983 & Kobayashi *et al.*, 1986) confirmed this pattern. Studies on multispawners (Bradford & Taylor, 1987; Greeley *et al.*, 1988; Kobayashi *et al.*, 1988) have shown multiple peaks of E-2 correlated with vitellogenesis but remaining relatively high even during oocyte maturation, ovulation and spawning in contrast with the rapid decrease observed in single spawners prior to spawning. Peaks in GSI varying between 3.5 and 30 (de Vlaming *et al.*, 1984; Bradford & Taylor, 1987; Everson *et al.*, 1989; Wilk *et al.*, 1990) have been shown to reflect ovarian maturation and the annual spawning cycle suggesting either a uni or bimodal spawning strategy. High values are normally indicator of fish producing only one batch per season while low values can indicate multiple or serial batches of eggs spawned over long period of time. With GSI values between 8 and 15, smallmouth bass compare with other summer spawners that can spawn more or less regularly during extended period of time. Wilk *et al.* (1990) reported some examples. Finally, constant summer conditions following a cyclic regime have induced a bimodal GSI curve in smallmouth bass (Fig. 5.2), which could be indicative of multispawning capability in smallmouth bass (Chapter 5).

Many studies have included measurements of total serum calcium over reproductive cycles. For example, in rainbow trout, Nagler *et al.* (1987) measured levels between 8

and 24 mg/dL for mature fish and 9 and 30 mg/dL for E-2 injected ones. Whitehead *et al.* (1978a), working with rainbow trout over a ten month period, found levels of 10-14 mg/dL from April to July, 33 mg/dL in October and 58 mg/dL in January, just prior to spawning. Booke (1964b) measured total serum calcium, in brook trout over a year cycle, finding a minimum of 5.5 mg/dL in August and a maximum of 27.8 mg/dL in November during the spawning period. Tinsley (1985) found plasma total calcium, in carp, to vary from 9.5 mg/dL to 41.1 mg/dL in E-2 treated fish. Olivereau & Olivereau (1979) reported plasma total calcium, of eel, to vary between 3 mg/dL to 57 mg/dL for E-2 injected fish. Sandstrom (1989) reported total plasma calcium, of perch collected over a year period, to vary from 10.8 mg/dL to 14.1 mg/dL prior to spawning time (March) with an increase in variance before and during the spring spawning. Based on blood sampling of smallmouth bass in New York State from June to November, Shell (1961) found serum calcium to vary from 10.2 at the end of August to 19.5 mg/dL in November. Data obtained, in the present study, fell within this range and confirmed the cyclic pattern observed by Shell (1961).

Animals having a seasonal breeding pattern generally also show a seasonal variation of vitellogenin levels in the bloodstream (van Bohemen & Lambert, 1981; Giorgi *et al.*, 1982; Scott & Sumpter, 1983b; Norberg *et al.*, 1989) as well as in calcium which peaks around spawning time. Results from the control tend to confirm that calcium levels increased with the vitellogenic activity observed in Autumn. The constant conditions that maintained this vitellogenic activity for longer period of time, had the same effect in maintaining high levels of plasma calcium. Atresia seemed also to affect calcium levels in a rather erratic way. This might have been caused by the rapid turn-over of certain stages of atresia, particularly the late stages where possibly the calcium bound lipoproteins are catabolized and reabsorbed into the bloodstream. It is not clear from the present study if the increase in plasma calcium assessed the vitellogenic activity and/or the atretic processes. However, total plasma calcium can remain an interesting and statistically significant indicator of vitellogenic activity, particularly when coupled with histology.

Wide variations have been reported in serum protein concentrations in fish (Alexander, 1977). Although the average protein concentration in teleosts is usually 4.0-5.0 g/dL, values between 2.9 and 12 g/dL have been reported for salmonids. Some authors found differences between sexes, being higher in females for brook trout (Booke, 1964b) and rainbow trout (Snieszko *et al.*, 1966), but not different in brown trout (Ingram & Alexander, 1977). These studies did not take into account the time of the year when the fish were collected neither their level of sexual maturity nor their nutritional status. Differences between strains of rainbow trout and diet have also been reported (McCarthy *et al.*, 1973). Shell (1961) found concentrations ranging from 1.8 to 4.5 g/dL for smallmouth bass collected from June to November. The lowest concentration occurred in August and was increasing. Under natural cyclic conditions, this would be a normal pattern associated with the onset of vitellogenesis.

Total proteins were higher in this study than those found by Shell (1961). Better nutrition with high protein feed can probably explain, in part, the higher levels observed. However, if results from groups kept under constant winter conditions were not included, the maximum levels recorded would have been only 6.1 g/dL and associated with important atresia (August-September). Again, variations in protein levels could probably indicate vitellogenic activity as well as atretic processes. Shell (1961) did not find any statistically significant correlation between calcium and serum protein as results presented here did. His data came from a pooled sample of serum from probably males and females, mature and immature fish which may explain the difficulty to correlate calcium and protein levels without sex or maturity consideration.

In the summer spawner channel catfish, MacKenzie *et al.* (1989) found no change in plasma total protein from September to March but then showed greatest variability throughout spawning season and summer with significantly higher levels in postspawning animals in July. No mention of atresia was made; however, high levels in plasma total proteins might represent atretic processes. Minimum values of total protein have also been reported at spawning (Lenhardt, 1992) and correlated with maximal

values of gonadal weight (Miller *et al.*, 1983). Similar results were recorded for smallmouth bass under natural conditions.

Peaks of E-2 during the active phase of vitellogenesis varied from 5 ng/ml in previously immature rainbow trout (Bromage *et al.*, 1982a), to 9.4 ng/ml in E-2 implanted brook trout (Lessman & Habibi, 1987), to 15 ng/ml in chum salmon (Ueda *et al.*, 1984), to 18-20 ng/ml in sockeye salmon (Truscott *et al.*, 1986), to 30 ng/ml in rainbow trout (Schulz, 1984). Annual cycles of plasma levels have been shown to vary from 1-10 ng/ml in the goldfish (Schreck & Hopwood, 1974), to 32-162 ng/ml in the plaice (Wingfield & Grimm, 1977) and 1-60 ng/ml in the rainbow trout (van Bohemen & Lambert, 1981). The peaks occurred, for these species, 1-3 months prior to spawning. Minimal and maximal levels recorded, in the present study, were 10 and 1568 pg/ml, respectively, with the highest level observed during the spawning period or associated with ovarian maturation. These levels appeared to be low when compared with salmonids but reflected lower levels found in species with asynchronous ovarian development (Pankhurst & Conroy, 1987; Rinchard *et al.*, 1993) and with synchronous ovarian development (Pankhurst & Conroy, 1988). The variations in concentration of E-2 agreed with the stages of maturity of the ovary found in the histological sections (Chapter 4) and the low levels of E-2 with the pattern of ovarian development, asynchronous for some individuals and group-synchronous for the majority.

The absence of dietary ascorbic acid has been shown (Waagbo *et al.*, 1989) to reduce serum levels of E-2 and vitellogenin in rainbow trout. Precautions were taken to provide a balanced diet for smallmouth bass, with daily supplementation of vitamin C and a weekly meal of natural food. Whitehead *et al.* (1978a) measured 130-4800 pg/ml in female rainbow trout during a simulated natural photoperiod cycle and a maximum of 3725 pg/ml under a constant photoperiod regime. Prat *et al.* (1990) measured 2-4.9 ng/ml (2000-4900 pg/ml), in seabass, with post-spawning levels of less than 1 ng/ml (1000 pg/ml). The peaks occured in the middle of spawning period and mid-summer. No explanations were given for this bimodal pattern. Seabass do have the capability of spawning more than once during the spawning season; a rise in E-2 during summer

could be indicator of the bimodal spawning strategy. Prat *et al.* (1990), however, did not correlate E-2 levels with the oocyte stages. In the case of smallmouth bass, the E-2 oscillations over 1 ng/ml detected with the presence of several clutches of vitellogenic and atretic oocytes could indicate a multispawning pattern (Chapter 4). Methven *et al.* (1992) observed, in the Atlantic halibut, a batch spawner, high levels of E-2 in females during gonadal recrudescence with lower levels during spawning. They suggested that levels of E-2 might be related to cycles of oocyte clutch development as shown for other fish (Kagawa *et al.*, 1983; Burke *et al.*, 1984; Kobayashi *et al.*, 1988). Rinchard *et al.* (1993) found high levels of E-2 in gudgeon during the gonadal regression and suggested that they were probably produced by preovulatory atretic follicles as in goldfish. In smallmouth bass, where the presence of atresia indicative of gonadal regression occurred, high levels of E-2 (0.4-1.6 ng/ml) were observed independently of the GSI or the presence of vitellogenic oocytes. Preovulatory atretic oocytes may play a role in maintaining E-2 levels in smallmouth bass as it was suggested by Rinchard *et al.* (1993) for the gudgeon. Finally, constant conditions, batch spawning pattern and intraspecific differences may explain the absolute low values of E-2 found in smallmouth bass.

Vitellogenin has been established as a major plasma protein in female salmonids close to sexual maturity (Copeland *et al.*, 1986). In mature eels, Hara *et al.* (1980) found a strong protein band concentrated in the β-region whereas in the male, the serum was rather diffusely distributed from albumin to γ-region, supported by purification techniques, it was later referred to vitellogenin. If this also applies to smallmouth bass, one would expect an increase in the β band either when fish are attaining sexual maturity or when induced with E-2. E-2 injections (Section 5.5.3) resulted in a peak in the β band as well as an increase in plasma calcium. In addition to the rapid increase in total serum protein, Campbell & Idler (1980) observed, in the rainbow trout, that E-2 treatment increased one band of protein at the expense of other proteins to the extent that faster migrating proteins were strongly reduced. Smallmouth bass electrophoretic profiles (Plate 5.2) showed the same pattern with a triple peak associated with the α_1 and α_2 bands and a reduction of the albumin band. These results do not agree with the findings of van Bohemen *et al.* (1982a) who reported that plasma total protein did not

display a clear response to oestrogen treatment. They mentioned though that their controls had a much higher protein levels than results from their previous study (van Bohemen *et al.*, 1981) without being able to explain fully the difference. It is obvious that total protein levels are only one possible indicator of a higher rate of vitellogenin transport and that it needs to be coupled with other indicators to be interpreted correctly. Higher protein intake (Lysak & Wojeik, 1960) has been shown to interfere with results.

Hepatic changes associated with oestrogen-induced hepatic vitellogenin synthesis included an augmented rate of protein synthesis (de Vlaming *et al.*, 1977; Olivereau & Olivereau, 1979; Medda *et al.*, 1980; Ng *et al.*, 1984) and enhanced calcium and phosphate metabolisms (Ho, 1991). Elevation of plasma calcium and protein levels in response to E-2 injection has been reported in *Mirogrex terrae-sanctae* (Yaron *et al.*, 1980), in carp (Tinsley, 1985) and in rainbow trout (Flett & Leatherland, 1989). The same pattern has been observed in smallmouth bass confirming, once again, the role of E-2 in proteogenesis related to vitellogenic activity. The fact that plasma protein levels continued to increase even after plasma E-2 levels had begun to decline suggests that once vitellogenin synthesis is initiated by E-2, the process continues to operate without an E-2 requirement and that other factors are involved in the termination of vitellogenin secretion. This would confirm an earlier observation of Bromage *et al.* (1982a) who suggested that once vitellogenin synthesis was initiated by E-2, it continues despite the return of E-2 to basal values. It is also complicated by the fact that if vitellogenin is not sequestered by the ovary, then it may remain in the plasma for many months. Successive administration of oestrogen or injection after a vitellogenic response has subsided has also been shown to produce a stronger and faster response than its predecessor in the singi fish (Sundararaj & Nath, 1981) and the flounder (Korsgaard *et al.*, 1983). This phenomenon, referred to as the "memory effect" of oestrogen has been related to irreversible increase of the number of hepatic oestrogen receptors, thus enhancing responsivity (reviewed by Wallace, 1985; Ho, 1991).

The oestrogen-induced vitellogenesis was performed in the spring (May-June) with fish from groups B2 and C2. At that time, total plasma calcium and protein were already

increasing in group B2 whilst they were decreasing in group C2. A possible explanation could be that under summer conditions fish were in a preparatory phase conducive to vitellogenesis whilst under winter conditions they were in a post-vitellogenic phase. These differences did not affect the pattern of response but rather the absolute value. From the results, it appeared that the increase of plasma calcium and total proteins levels were controlled by E-2 levels whilst their rate of utilization was probably regulated by temperature (Chapter 6). E-2 treatments have shown to induce vitellogenin and an additional protein in striped bass (Kishida *et al.*, 1992). The increase in the β band and the triple peak associated with the α_1 and α_2 bands observed in the electrophoretic profiles of smallmouth bass could be related to the lipo and glyco-proteins associated with the vitellogenin. Further studies would be necessary to ascertain directly this shift in the composition of proteins.

Oestradiol implants (Lessman & Habibi, 1987) have been reported to produce asynchronous development and loss of clear-cut clutch progression in brook trout ovaries. They reported low, constant levels of plasma E-2 (about 3 ng/ml) and postulated that E-2 may influence recruitment of small oocytes into clutches and elicit asynchronous growth. The *in vivo* involvement of E-2 in ovarian function of fish has been considered indirect except in partial maintenance of yolky oocytes in hypophysectomized catfish and cortical alveoli formation in hypophysectomized goldfish oocytes (Fostier *et al.*, 1983). E-2 can depress the gonadotropin which in turn may affect vitellogenin uptake, decrease oogonial cells as well as the smallest size oocyte and increase atresia (Tam *et al.*, 1983). Asynchronous development, loss of clear-cut clutch progression, low levels of E-2, maintenance of yolky oocytes were all observed under the constant regimes. Using constant temperature, Lessman & Habibi (1987) started in the spring when fish ovaries were developing; the present study started in autumn when smallmouth bass ovaries were entering into their vitellogenic phase. As they pointed out, the timing can affect the response obtained. Timing is certainly of prime importance in modifying any physiological state but the fact remained that what they explained as the effects of E-2-implant on the ovarian development has also been observed in individuals kept under constant conditions. How constant temperature and E-2 levels intervene is

not that clear; however, some *in vitro* studies (Andersson *et al.*, 1992) have suggested a pathway (Chapter 6).

Finally, stress can also have affected individual smallmouth bass reproductive physiology. Whittier (1991) mentioned "wild species retain genetic diversity and retain sensitivity to stress and other environmental challenges". She gave evidence that stress may collectively induce a decrease in the expression of sexual behaviour and via the corticosterone pathway and may act to decrease secretion of GnRH and sex steroids. Greenberg & Wingfield (1987), in their review on stress and reproduction, reported a role for cortisol in lowering the threshold to maturation-inducing factors. Evidence of stress reactions were not investigated in this study, but nevertheless further experimentation on confined and constant conditions as stressors might provide insight on the fish response to modifications of its environment.

SUMMARY

Gonado-somatic index (GSI), relative condition factors (RCF) and plasmatic components varied with the ovarian development. GSI and RCF peaked prior to spawning and reflected the levels of fish maturation. Total plasma calcium correlated positively with the percentage of vitellogenic oocytes and levels of oestradiol-17β (E-2). Total protein correlated positively with total plasma calcium; levels of proteins were at their lowest during summer, but the absolute values were always greater in fish kept under constant winter conditions. The electrophoretic profile of protein indicated a peak between the α-1 and α-2 bands associated with fish having high levels of E-2; an increase in the β band was associated with high levels in total plasma calcium. The reduction in peaks of E-2, as a result of constant conditions, may be responsible for the asynchronous development, the loss of clear-cut clutch progression and the maintenance of yolky oocytes reported in Chapter 4. Rates of utilization of E-2, total plasma calcium and total protein subsequent to E-2 injections showed dependence on temperature.

CHAPTER 6

INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON THE REPRODUCTIVE CYCLE OF SMALLMOUTH BASS

6.1 Introduction

There is extensive work on the use of controlled daylength cycles to accelerate maturation, advance spawning time and induce gonadal recrudescence in temperate and cold water teleosts (see reviews by Billard, 1983; Billard & Breton, 1985; Abraham, 1988; Bromage *et al.*, 1990). There is also work using constant photoperiod or temperature to control spawning in rainbow trout (see review by Bromage *et al.*, 1992b) and in the gilthead bream (Micale & Perdiccizzi, 1988) and to delineate the endogenous rhythm in the catfish (Sundararaj *et al.*, 1973; Sundararaj & Vasal, 1976), brook trout (Henderson, 1963), dab (Htun Han, 1975), rainbow trout (Scott & Sumpter, 1983a) and stickleback (Baggerman, 1980). Temperature manipulation has also been used to control gametogenesis (deVlaming, 1972a), to stimulate hormonal responsiveness of the gonad (Jalabert *et al.*, 1977) and the production of vitellogenin in response to oestrogen treatment (Yaron *et al.*, 1980), and more recently to influence hormonal release from the hypophysis and the pineal gland, respectively (Andersson *et al.*, 1992; Zachmann *et al.*, 1992). In most cases, emphasis has been selectively put on either temperature or photoperiod as the animals may not integrate synergistically the information available. This has resulted in mixed responses particularly when photo-thermal history has not clearly been defined. The synergy between temperature and photoperiod, the photo-thermal history as well as the response to constant conditions were the important considerations of this study.

6.2 Effects of temperature and photoperiod on ovarian development

Ovarian development of smallmouth bass was influenced by the different regimes of temperature and photoperiod. The changes in oocyte diameter (Fig. 6.1) for all conditions indicated that larger vitellogenic oocytes were present and maintained under a cold temperature-short photoperiod regime. Simultaneously, atretic oocytes became

dominant particularly during the second year of exposure to constant winter conditions and independently of season. Summer conditions did not promote progression of vitellogenesis unless fish had encountered winter condition during the preceding year (group A1), and the average size of the oocytes remained small. Clearly, a pattern of oocyte growth followed by a reduction in size, a pattern repeated up to three times per year, was seen in groups A2 and D2. In group B2, summer conditions slowed the vitellogenic activity while in C2, winter conditions promoted it.

The GSI was maintained low under warm temperature-long photoperiod conditions, while it increased to reach and to stay at pre-spawning levels under the cold temperature-short photoperiod regime. Under natural and compressed cycles, oocyte growth, particularly vitellogenesis, was related to cold temperature-short photoperiod followed by a diminution in size as the mature oocytes were released. Pre-spawning and spawning GSI were comparable to the peaks measured under constant cold temperature-short photoperiod regime.

Intraspecific differences, in terms of asynchronous or group synchronous ovarian development, were observed. Pre-and vitellogenic oocytes without clear-cut differences in oocyte sizes were found in a minority of individuals under both constant regimes. Vitellogenic activity, maturation and atresia were observed in fish maintained under constant short photoperiod cold temperature regime, while vitellogenic activity, no maturation and limited atretic processes were observed under constant long photoperiod warm temperature regime.

6.3 Effects of temperature and photoperiod on plasmatic components

The effects of temperature and photoperiod on total plasma calcium (Fig. 5.8) indicated a relatively stable concentration in the plasma of fish kept under summer conditions (B2) while there was a gradual increase peaking around what would have been the normal spawning period (May-July) in group C2.

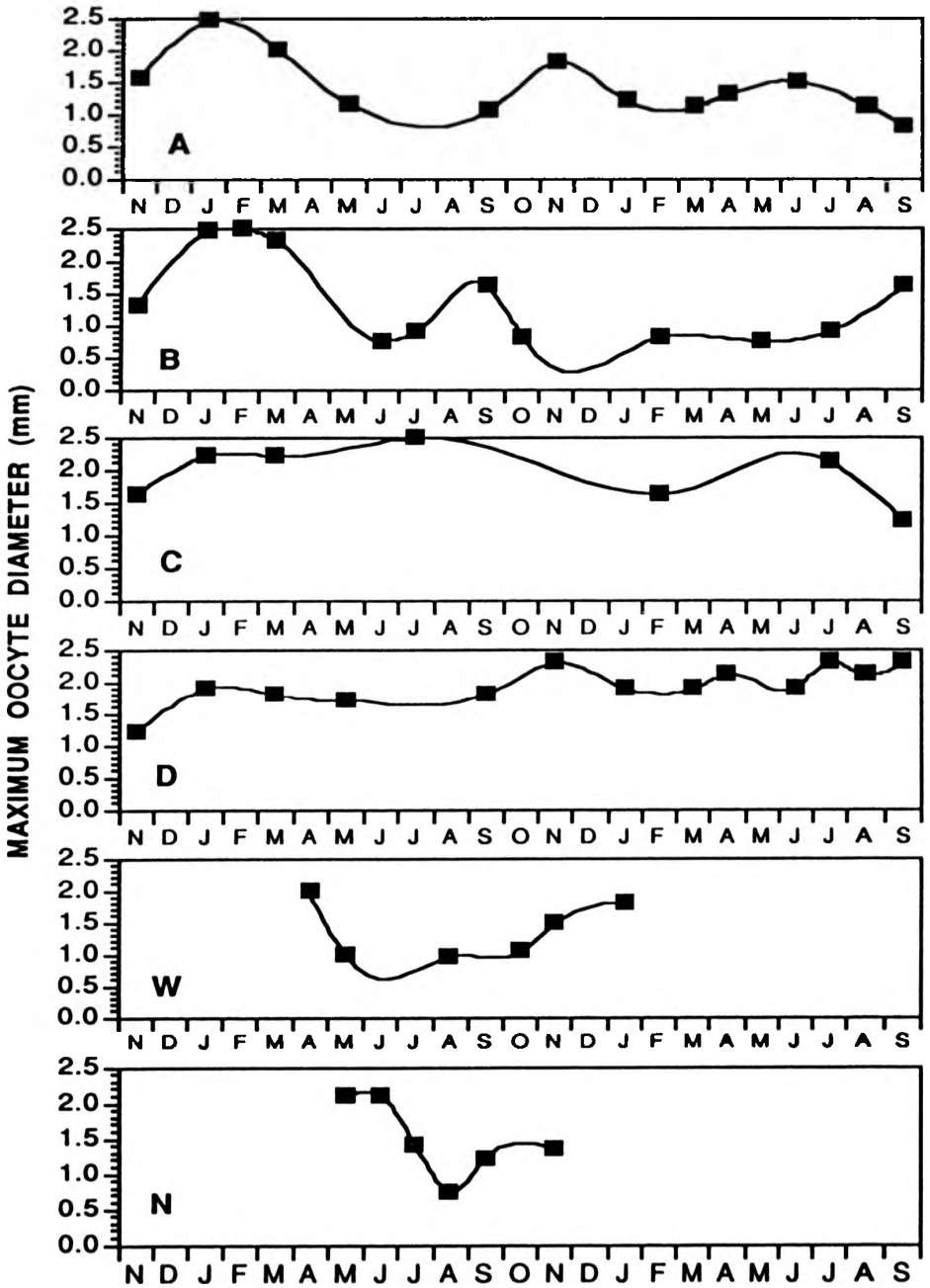


Fig. 6.1 The pattern of changes in oocyte diameter over two years of constant 15L:9D, 22°C regime: groups A1 and A2 (spawners), group B1(missed last spawning), group B2 (first time spawners); constant 9L:15D, 10°C regime for groups C1 and C2 (spawners), group D1 (missed last spawning), group D2 (first time spawners); seasonal regime with a compressed winter (2 months) to induce an early spawning (January-February) in group W, and a seasonal regime found at 45°N, 71°W, for group N.

Long term exposure to constant photoperiod-temperature regimes, particularly winter conditions, resulted in an increase in plasma total proteins probably as a result of a high rate of atresia. The electrophoretic profile of smallmouth bass indicated that the high level of protein observed was mainly due to the presence of a peak located between the α_1 and α_2 band. Elevated levels of plasma proteins can be related to vitellogenic activity, anabolically as well as catabolically. Results from the histology (Chapter 4) indicated both processes.

When comparing GSI, calcium levels and E-2 levels between the two regimes, the constant long photoperiod-warm temperature regime maintained a low GSI (<2), while under the short photoperiod-cold temperature regime the GSI reached 15.19 in July. E-2 levels were in the same range of values (220 - 1568 ng/ml) independently of the regime and showed peaks between April and July. The delayed peak in July was observed in fish under short photoperiod-cold temperature regime. Calcium levels were generally lower in fish kept under short photoperiod-cold temperature regime.

6.4 Effects of temperature and photoperiod on the timing of spawning

Results described in Chapter 3 indicated that smallmouth bass can spawn during short or long photoperiod and at temperatures between 14°C and 25°C with a peak around 18°C. Natural spawning occurs during spring up to early July when temperatures normally oscillated between 15°C and 22°C but stops when water temperatures approach 24°C.

Smallmouth bass, kept under summer constant conditions for the first time, were ready to spawn three months before normal spawning time while the ones kept under winter constant conditions were delayed for about the same amount of time. A second year of exposure to constant winter conditions yielded individuals ready to spawn at any month of the year. Extended spawning time, controlled spawning time and lack of intraspecific synchronization were all observed under constant temperature-photoperiod regimes.

It is particularly noteworthy that individuals from groups B2 and/or C2 left under their respective conditions for 10 months after the end of the experiment, then transferred into natural conditions at the end of July, spawned from mid-August to early September. Water temperatures were between 15-20°C, and photoperiod was decreasing from 13L:11D to 12L:12D.

6.5 Discussion

To summarize the results under constant conditions, cold temperatures (around 10°C) were necessary to maintain normal ovarian development and to delay spawning; an increase in temperature was necessary to stimulate ovulation and spawning; warm temperatures limited vitellogenic activity and short or long photophase did not affect ovulation nor spawning. Constant conditions desynchronized ovarian development indicative of smallmouth bass' endogenous rhythm. Under natural conditions, decreasing photoperiod might have triggered the onset of vitellogenesis maintained by decreasing temperature followed by the increase in temperature (between 15° and 20°C) to trigger spawning. It would then appear that temperature plays a crucial role in maintaining the integrity of the developmental processes while photoperiod may help reset them in accordance with the environment. Constant conditions had an impact on the intraspecific synchronization of oocyte development, independently of the temperature and photoperiod regimes tested. This was particularly evident during the second year of constant conditions.

Successful spawning, in smallmouth bass, is dependent on the presence of cold temperatures (10°C and less) during the preceding year (Chapter 3). Robbins & MacCrimmon (1974), in their study of ecological factors associated with the success of introductions of smallmouth bass throughout the world, suggested that water temperature is the most important single factor limiting distribution. While warm temperature is essential to promote rapid growth of the fingerling, so they can attain sexual maturity before excessive predation limits the population, the presence of colder temperature can be as essential to promote ovarian development, particularly maturation. Previous results have shown that two months of winter conditions were

sufficient to induce final maturation and resulted in early spawning as soon as spring conditions were present (Cantin, 1988). Also, extended periods of winter conditions have delayed spawning up to mid-July (Cantin, 1988).

Temperature is a master factor affecting the rates of processes. In his review of the effects of temperature on fish, Brett (1973) summarized the intricacies of temperature. It may act as a controlling factor setting the pace of metabolism and development, as a limiting factor restricting activity and distribution, as a masking factor interacting with other environmental entities by blocking or altering their potential expression, and finally by providing a directing agent as a gradient stimulating sensory perception and oriented response. The effects of temperature can be relative when associated with other factors or absolute if defining the limits for any biological activity. For example, Kinne (1963) reported that temperature appears to confine spawning to a narrower range than the majority of other functions. Lee & Hirano (1985) showed that temperature was the most important factor in initiating and terminating the spawning season. Temperature has been determined as the most important factor in regulating gonadal development/maturation and/or spawning in several species such as goby (de Vlaming, 1972a), mullet (Kuo *et al.*, 1974), sand borer (Lee & Hirano, 1985), African catfish (Richter *et al.*, 1987), Chinese catfish (Young *et al.*, 1989), carp (Gupia, 1975; Bieniarz *et al.*, 1978; Davies *et al.*, 1986) and catfish (Pacoli *et al.*, 1985). Water temperature has been shown to be an influencial factor on spawning time of rainbow trout (Nakari *et al.*, 1987). Rainbow trout (Bromage *et al.*, 1992b) maintained under constant and seasonally varying temperatures experienced advances in spawning time in response to a stimulatory photoperiod. In many freshwater species, water temperature seems to be an important physical factor influencing the initiation of spawning (Rodriguez-Ruis & Granado-Lorencio, 1992). The reproductive cycle of smallmouth bass, particularly the spawning activity, is also dependent on the presence of specific temperatures, in this case around 18°C, to occur. The regulation of spawning activities in smallmouth bass is certainly highly dependent on specific temperature.

Ovarian recrudescence is a function of warm temperature in many species, particularly in Indian catfish (Sundararaj & Vasal, 1976), channel catfish (MacKenzie *et al.*, 1989) and catfish (*Clarias macrocephalus*) (Pacoli *et al.*, 1985). Busch & Steeby (1990) showed that induced spawning of channel catfish yielded better results at warmer temperatures, thus indicating an effect of temperature on final maturation and ovulation. Davies *et al.* (1986) showed that cool temperature induced rematuration in carp, and increasing temperatures up to 25°C combined with long photoperiod induced spawnings at any time of the year. Similar to these species, variations of temperature were directly responsible for alterations in the reproductive cycle of smallmouth bass. Gonadal recrudescence has been stimulated by a drop in temperature (15°C) followed by an increase (20°C) over a two week cycle (Cantin, 1987a). Simultaneously, photophase was either constant long, short and/or increasing (15D:9L) or long and/or decreasing (9D:15L). Most steps appeared to be temperature dependent, from the vitellogenic activity to the atretic processes and the initiation to the termination of spawning. This could explain, in part, the limited distribution of smallmouth bass which needs specific temperatures to spawn, colder temperatures to mature and warmer temperature to rest and start the next ovarian cycle.

De Vlaming (1972a) mentioned that at minimal temperatures of 24°C, vitellogenesis is inhibited and gonadal recrudescence does not occur, in the longjaw goby. High temperatures retarding the early phases or intermediate phases has been documented in *Fundulus confluentus* (Harrington, 1959), *Apeltes quadracus* (Merriman & Schedl, 1941) and *Leuciscus cephalus* (Poncin *et al.*, 1989). Limited vitellogenesis and the absence of maturation were observed in smallmouth bass kept at constant 15L:9D, 20°C for a second year. Furthermore, Tyler *et al.* (1987) showed, in rainbow trout, that vitellogenin uptake was dependent on temperature with greater amounts of proteins being sequestered as the temperature increased. Lower levels of plasma proteins found in smallmouth bass maintained under warm temperature (20°C) could also indicate an effect of temperature on protein uptake as temperature increases. Long exposures to high temperature in goldfish stimulated gonadotropin secretion (Gillet *et al.*, 1978). Andersson *et al.* (1992) showed that the gonadotropin-releasing hormone binding

(GnRH) in the pituitary was undetectable at low temperature. This would suggest that levels of circulating gonadotropin (GtH) in the blood would be low possibly affecting vitellogenin uptake induced in rainbow trout, by GtH I (Tyler, 1991) and via the GtH I regulation of follicular oestradiol established for salmonids (Swanson, 1991). In female salmonids (Crim *et al.*, 1975), increases in GTH were associated with increased vitellogenic activity. Increases in temperature would then affect vitellogenic activity. This might apply only to the range of temperatures below spawning temperature in smallmouth bass. Temperatures above the range of spawning temperature might affect vitellogenic activity in a different way. In most spring and summer spawners, increasing the water temperature induces vitellogenesis and spawning (Breton *et al.*, 1980). At high temperature, gonadal recrudescence was enhanced only at long photoperiods, but at low temperatures, it was enhanced regardless of photoperiods in the cyprinid *Gnathopogon caerulescens* suggesting that the factor initiating gametogenesis is low temperature (Okuzawa *et al.*, 1989). In the green sunfish, Kaya (1973) showed that gonadal recrudescence occured under increasing temperature and that responsiveness of pre-spawning ovaries to pituitary hormones was markedly influenced by temperature. There was no response at 10°C. Kaya (1973) suggested that cold temperature could inhibit gonadotropin production. Temperature may have an effect on the rates of synthesis and/or utilization of oestrogen, but the timing of the temperature regime on the physiological state of the animal can be decisive. Data on E-2 levels collected from smallmouth bass did not reveal direct influence of temperature. However, testosterone and progesterone levels were not sampled during this study. Progesterone, in many oviparous species is secreted by the granulosa cells of the follicle, controls the process of oocyte maturation (Selcer & Leavitt, 1991) and is generally considered a modulator of oestrogen activity in vertebrates (Leavitt *et al.*, 1983). Atresia was quantitatively important under many conditions and may have participated in progesterone secretion. Testosterone is produced by thecal cells of the oocyte and then transferred to the granulosa cells to be aromatized to oestrogens (Kagawa *et al.*, 1982) and could follow the two cell-type model proposed by Nagahama (1983). The presence of vitellogenic oocytes at the same time as atretic oocytes under constant conditions might have induced antagonistic activity of oestrogens and

progestogens and amplified the effects. Further studies could include monitoring levels of testosterone to verify if the testosterone was aromatized to oestrogens. Progesterone monitoring could provide information as to whether it had limited the oocyte maturation which did not occur under constant 15L:9D, 20°C conditions.

In their study of the effects of photoperiod and temperature on serum proteins in rainbow trout, Meisner & Hickman (1962) did not show any significant effect of photoperiod but found a decrease in the β and α-2 globulins and an increase in albumin and α-2 fractions at 8°C, while the β fraction increased at 16°C. Increased β and γ fractions were observed in smallmouth bass kept under constant 9L:15D, 10°C conditions. Other fractions were correlated with levels of oestrogens and/or plasma calcium rather than environmental conditions. Seasonal modifications in the serum protein pattern (Saito, 1957) have been reported as a result of changes in water temperature and in post-ovulated rainbow trout due to the resorption of yolk during follicular atresia (Babin, 1987). In their studies on salmon, Olin & Von der Decken (1989) and Olin *et al.* (1989) reported an increase in protein synthesis with temperature correlated with a faster increase in vitellogenin subsequent to E-2 injection. Similar results were obtained with smallmouth bass and confirmed the effect of increased temperature increasing plasma proteins subsequent to E-2 injection.

Photoperiod has been reported to be the dominant factor in determining the breeding season of many species: MacQuarrie *et al.* (1978), Kuo *et al.* (1974), Baggerman (1972), Htun-Han (1975), Sehgal & Sundararaj (1970), Sagi & Abraham (1985), Bromage *et al.* (1984, 1988), Brook & Bromage (1988), Lee *et al.* (1987), Smith *et al.* (1991) and Kestemont *et al.* (1991) are among the extensive list. Decreasing photoperiod has also been reported to inhibit sexual maturity in the barbel (Poncin & Philippart, 1986; Poncin *et al.*, 1987). However, these spring spawners were submitted to decreasing photoperiod, near the end of the spawning period, and internal factors might already have started the process. Poncin (1989) showed that decreasing daylength inhibited the spawning, in the barbel. In 1990, the same author also reported a decrease of the average number of spawnings per female and shorter reproductive

periods after 3 years of constant short photoperiod and one year of constant long photoperiod. In their study of the bluegill, a centrarchid, Lee & Kim (1987) tested long (15L) and short (10L) daylengths with warm (25-28°C) and cool (15°C) temperatures on the growth of the ovaries. They found the combination 14L/25°C produced the greatest GSI during the resting and the vitellogenic periods, and 10L/20-28°C reduced the GSI. They concluded that long photoperiod are necessary to activate the gonads and shorter ones to terminate the breeding season while temperature was responsible for regeneration. Yaron *et al.* (1980) showed the highest GSI was found in fish that had been exposed to a simulated winter regime, and the lowest GSI was in those exposed to a summer regime. The maximal GSI was also found in smallmouth bass exposed to a constant winter regime while low GSI with high E-2 and calcium levels was recorded in September in smallmouth bass kept at constant 15L:9D, 20°C conditions. The GSI is an index of ovarian growth and maturation and corroborates with the lack of oocyte maturation observed in smallmouth bass exposed to constant 15L:9D, 20°C conditions.

In the seabass, constant long days delayed maturation and spawning time by 2-3 months, while constant short days advanced spawning by up to 6 weeks (Carrillo *et al.*, 1989). They also found that different light cues are not required for the completion of reproduction, for spawning occured with fish maintained under constant short days. Constant temperature (10°C), with constant long days early in the year (February) in a January spawning group of rainbow trout, advanced spawning by 6 months (Bromage *et al.*, 1984). In some instances, combinations of long photoperiod and warm temperature have been shown to be essential in stimulating spawning in the green sunfish (Kaya & Hasler, 1972) and in *Notemigonus crysoleucas* (de Vlaming, 1975). Rainbow trout normally reproduce once a year over a 6-8 week period (Kato, 1973) with the exception of certain strains which reproduce biannually (Lou *et al.*, 1984). Some strains spawn in Autumn while others spawn in Spring. Multi-annual spawning has often been induced by photoperiod manipulation using altered seasonal light cycles or constant cycles (Bromage & Duston, 1986). Scott *et al.* (1984) using constant long daylengths induced rainbow trout to spawn at 6 month interval. Constant long

photoperiods have also been reported to either delay spawning (Allison, 1951) or advance spawning (Whitehead & Bromage, 1980). The importance of the natural spawning time of the fish and the time of the year the fish were subjected to constant conditions have also been thoroughly discussed (Scott *et al.*, 1984). However, the distinction between constant and cyclic conditions did not receive as much attention. For instance, in the gilthead bream, constant temperature-photoperiod regime (16L:8D; 21°C) contributed to abnormality in the phases of gonadal development alternating with periodic atresia (Kadmon *et al.*, 1985). In rainbow trout, Bromage & Duston (1986) mentioned that constant year-long light regimes yielded less synchronized spawnings than those produced by seasonal cycles. Furthermore, the work on the barbel (Kestemont & Philippart, 1991) suggested that a fish can modify its reproductive strategy from group-synchronous to asynchronous type of development in response to the environment. The synchronization of reproductive processes in smallmouth bass was modified by constant conditions, and some individuals displayed asynchronous type development in response to an unseasonal pattern of temperature and photoperiod. This would suggest that within a population, individual responses can have an evolutionary impact and also decrease the year-to-year variations of number and timing of spawned eggs.

Irregular spawning has been reported by Kjesbu (1989) for captive cod maintained at constant temperature during their spawning period. Stress was believed to be the cause. Stress can affect levels of cortisol which in turn has been shown to enhance follicle sensitivity to gonadotropin and 17α-20β progesterone (Jalabert, 1976). In the winter flounder, Tyler & Dunn (1976) reported 58% of females with yolk-bearing ovaries under constant temperature compared to 100% for fish maintained under natural cycling conditions (Burton & Idler, 1987). In a study on the Chinese catfish, Young & Fast (1990) showed that constant warm temperature (25°C and 30°C) maintain fish in spawning condition year round while they would normally spawn from May through September under ambient conditions. The same way, constant winter regime maintained smallmouth bass in prespawning conditions over a long period of time. Similarly, female lobster are capable of spawning well before their normal summer

spawning period and an extended period of cold water can force the animals into a waiting period before the onset of final vitellogenesis and spawning (Waddy & Aiken, 1992). Resorption of oocytes or lengthening the post-spawning stages due to unfavourable conditions of the habitat can interfere with development of next year batch of oocytes in fish with synchronous oocyte growth like the perch (Hokanson, 1977); under such conditions an asynchronous type ovary would then become an advantage.

Rainbow trout (Randall *et al.*, 1991a,b) maintained on constant daylength exhibited a desynchronization of spawning times characteristic of a free-running circannual rhythm. The results also confirmed that rainbow trout reads daylengths comparatively, with reference to the preceding photoperiod, rather than absolutely. Many studies (Asahina & Hanyu, 1983, 1985; Bourlier & Billard, 1984; Asahina *et al.*, 1985) indicated that the response of the gonad to photoperiod varied with the season and the temperature. The *in vitro* experiment of Zachmann *et al.* (1992) partially explained how by showing that low temperature slightly reduced release of melatonin over time. This would suggest that the pineal gland can function as a transducer of both thermal and photic information to regulate seasonal activities, as for example, reproduction. The effects of photoperiod on the time of spawning are confirmed by changes in the levels of E-2 and total calcium in the rainbow trout (Duston & Bromage, 1987). Bromage *et al.* (1982a) showed that constant photoperiod produced a similar sequence of changes of gonadotropin, E-2 and vitellogenin as seen under natural seasonal cycles. Johnston *et al.* (1987) observed a decline in plasma protein levels, in salmon, following ovulation. It has been shown that total E-2 concentration, in rainbow trout, can be reduced by 52% (5000 to 2400 pg/ml) by altering photoperiod cycles (Bromage *et al.*, 1982a); the timing of the E-2 peak, however, was not affected. In a previous study, Whitehead *et al.* (1978a) comparing constant conditions with seasonal cycles demonstrated the same pattern; the peak had reduced values (1000 pg/ml less than 12 month cycle) for fish kept under constant conditions. Non-cycling photoperiod-temperature regimes may contribute to the reduction of E-2 production.

Temporal integration, in fish involving multistimuli (photoperiod, temperature and feeding cycle) entraining externally expressed rhythms, has not been frequently studied (Weber & Spieler, 1987). MacGregor III *et al.* (1985) suggested that feeding cycles of killifish rather than light-dark transition were a more important entraining agent of endogenous rhythms. Under constant conditions of light and temperature, Arctic charr, displayed distinct cycles of food intake (Jobling, 1987). Under constant temperature but seasonal photoperiod, De Silva & Balbontin (1974) also found distinct cycles of food intake in the herring. Long-term exposures to constant conditions of photoperiod, temperature and feeding rates (Duston & Bromage, 1986) resulted in early spawnings (2 months earlier than controls), a loss in synchrony of spawning between individuals suggestive of a "free running" rhythm and two yearly phases of gonadal activity.

Temperature and photoperiod are exogenous cues that can act in concert or independently in regulating the timing of spawning, as well as the many steps leading to spawning. While their use in the modification of the timing of spawning, however is widespread, their specific effect on oogenesis, vitellogenesis, maturation and ovulation is dependent on seasonal variation in sensitivity and possibly on the effects of stress caused by constant conditions. Nevertheless, the ovarian development of smallmouth bass has shown to be primarily dependent on specific temperatures to reach maturity and to terminate spawning while the impact of photoperiod seemed to be limited to the synchronization of the early phases of vitellogenesis. The ultimate factor for controlling spawning and proximate factor for controlling oocyte maturation, temperature can certainly limit directly the reproductive activities of smallmouth bass. The role of photoperiod as a proximate factor for controlling reproductive activities of smallmouth bass, besides helping them maintain their intraspecific synchronization, seems minor compared to temperature and is certainly indirect. Similar results were reported for siluroids, cichlids and some poeciliids (Munro, 1990) while in many cyprinids (Hontela & Stacey, 1990) photoperiod may enhance the role of temperature. In the mummichog, Day & Taylor (1984) suggested that photoperiod and temperature may influence different phases of ovarian function. Smallmouth bass should not be considered a "photoperiodic animal" when compared with most salmonids but rather

dependent of specific temperatures, confirming earlier works about its distribution in relation to seasonal variations of temperature (Chapter 3).

SUMMARY

Cold temperatures were necessary to maintain normal ovarian development and to delay spawning; increase in temperature was necessary to stimulate ovulation and spawning; warm temperatures limited vitellogenic activity and short or long photophase did not affect ovulation nor spawning. Constant conditions desynchronized ovarian development indicative of smallmouth bass' endogenous rhythm and multispawning capacity. Smallmouth bass reproductive cycle is dependent on the presence of cold (around 10°C) temperatures to attain oocyte maturation and spawning. Temperature is more important than photoperiod in regulating smallmouth bass reproductive cycle.

CHAPTER 7

CONCLUSIONS

Temperature has been shown to influence the reproductive cycle of smallmouth bass particularly in stimulating oogonial proliferation, in the maintenance of vitellogenic and atretic activities and in promoting spawning. Simultaneously, short or long photoperiods did not directly influence spawning nor any stage of oocyte development. Natural or protracted temperature-photoperiod regimes have led to synchronized ovarian development with clear-cut clutches of oocytes while, in some individuals, a random mixture of oocytes with a wide range of diameters was observed under constant regimes. This last type of ovary could be considered asynchronous and offers a certain advantage in anticipation of conditions promoting spawning. Natural spawnings from April to the end of September and induced spawnings from October to June have indicated that under controlled temperature and photoperiod regimes conducive of spawning, smallmouth bass can spawn at any time of the year. The same individual can also spawn many times at two week to a month interval, but all individuals do not necessarily spawn more than once. How often do they spawn during the same season can be an individual decision. Fecundities and nest productivity were highly variable but fell within a range of previous field studies and fish which display parental care for their off-springs.

There is extensive work on the effects of temperature and photoperiod directly on protein synthesis, liver function, hormonal, plasmatic components and brain chemical release/inhibition, oocyte development, ovulation, spawning and reproductive cycle. These exogenous factors show seasonality, importance of previous or acclimation conditions and interdependence. Bye (1990) reported that the photoperiodic control of maturation is weak, while temperature has a pronounced effect on spawning and the rate of gonadal development in fish. The results obtained with smallmouth bass agreed with those studies. However, the extensive studies using photoperiod and/or temperature to modify the reproductive cycle raised many other questions. Most often,

stress reactions related to captivity, handling, constant conditions or conditions near the limits of the natural range of the animal were hardly considered. Desynchronization of the ovarian development subsequent to long term exposure to constant conditions might be a response to a stressful situation where the perturbation of the reproductive cycle, in relation to previous cycles, prevents its use as a predictive cue. Asynchronous oocyte development would then be a response to the insecurity caused by the presence of abnormal conditions. This suggested that the fish can respond to a specific factor in an absolute and relative way (constant or cycling). The purpose of this research was to look at the responses of the ovarian development to constant and cycling temperature and photoperiod regimes. Stress has certainly played a part in the asynchronous ovarian development observed. However, the comparison between the different regimes indicated specific temperature/photoperiod effects. Specific temperature (around 18°C) is necessary to assure a successful spawning, the presence of cooler temperature for a limited period of time is necessary to promote oocytes maturation, and warm temperatures ($\geq$ 22°C) can inhibit oocyte maturation. Low temperatures have also maintained higher levels of total protein, total plasma calcium and E-2 by decreasing their rate of utilization. Decreasing, increasing, constant short or long photoperiods, all the regimes tested can allow a successful reproductive cycle for smallmouth bass. Constant conditions limited the peaks of all blood parameters studied.

This study confirms the statement of Robbins & MacCrimmon (1974) in regard to temperature limiting the worldwide distribution of smallmouth bass and defines the proximate and ultimate effects of temperature on the reproductive cycle of smallmouth bass. Further studies with this highly adaptable fish regarded as opportunistic and fully aware of its environment, could, for example, consider comparisons of the reproductive cycle of different populations at different latitudes, the role that atretic processes may play in controlling ovarian maturation under unfavourable conditions and the male-female synchronization at the time of spawning. The field of reproductive physiology could certainly benefit from continued studies on smallmouth bass.

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APPENDICES

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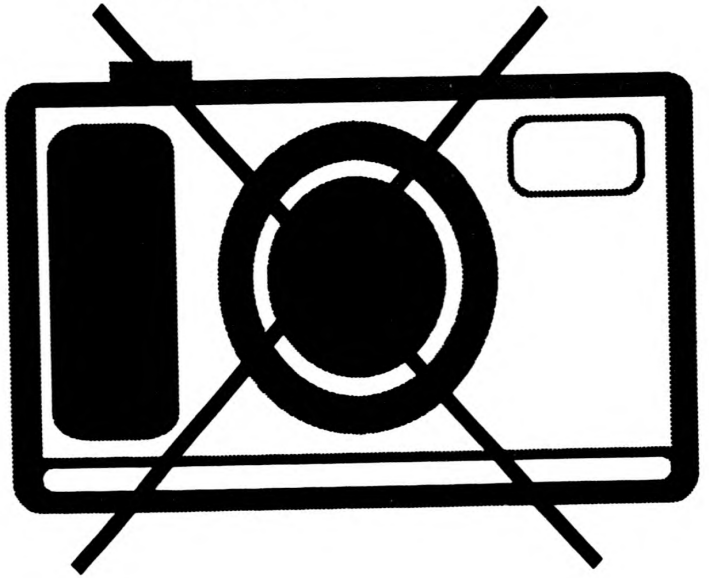

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Oocyte Development and Induced Spawning of Smallmouth Bass (*Micropterus dolomieui*) in Response to Photoperiod and Temperature Manipulation: Preliminary Results

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Abstract

Smallmouth bass (*Micropterus dolomieui*) in Quebec spawn between the end of May and mid-June. Wild broodstock (4 to 8 years old) and laboratory-reared ones (0+ years old) reared in a water recycling system were submitted to different temperature and photoperiod regimes to extend their natural spawning period. Oocyte maturation followed *in vivo* showed that oocytes developed to stage III in both cases but final maturation and ovulation was limited to the laboratory-reared bass and this, a month before the normal spawning period, at 20.5 cm TL. Exogenous factors are examined, specifically duration of long photoperiod and vital space related to nonspawning of wild broodstock.

Smallmouth bass (*Micropterus dolomieui*) are a sport fish with wide distribution in southern Quebec. Their restricted spawning season of about two weeks from the end of May to mid-June when temperatures rise to 16–18 C (Scott and Crossman 1974) limits development of larval rearing techniques and restricts the period for restocking. Several previous attempts to induce wild fish to spawn in a local governmental hatchery were unsuccessful (R. Thibault, personal communication). These efforts showed that smallmouth bass are particularly sensitive to manipulation which seemed to upset synchronisation between males and females (A. Fortin, personal communication).

Induced spawning by manipulation of photoperiod and/or temperature has been reported for various salmonids (Whitehead and Bromage 1980; Bromage et al. 1984; Scott et al. 1984; Sumpter et al. 1984; Takashima and Yamada 1984) but few experimental demonstrations are available on centrarchids (Carlson and Hale 1972; Carlson 1973; Kaya 1973). Results presented here pertain to effects of constant and cyclic photoperiods coupled with low temperature regimes on spawning, behaviour, oocyte maturation and ovulation of the smallmouth bass. Importance of key exogenous factors that may influence spawning induction is also discussed.

Materials and Methods

Wild broodstock smallmouth bass were captured in a local lake and acclimated in a water-recycling system with constant temperature (24 C) and photoperiod (18L:6D) for three months prior to experimentation. Laboratory-reared broodstock provided by Aquaresearch LTD were reared from larvae in a water-recycling system (Ehrlich et al. 1986) under constant temperature (24 C) and photoperiod (18L:6D). Wild and laboratory-reared broodstock were used in Experiments I and II, respectively. Water-recycling systems were used in all phases of this research. Temperatures were maintained to within ±0.1 C. Six fish (four females and two males), tagged to allow individual identification, were selected for each experiment and were fed dry food *ad libitum* throughout the experiments. Each spawning tank was provided with two gravel nests of 60 cm diameter with substrate ranging from 0.2 to 18 cm in diameter. Temperature, dissolved oxygen, pH, TAN, conductivity, NO_2-N and NO_3-N were monitored daily and iron, copper, hardness,

TABLE 1. *Total length (cm) and weight (g) of individual fish at beginning and end of experiments.*

	Experiment I			Experiment II	
	T.L. (cm)	Wt (g)		T.L. (cm)	Wt (g)
October 84	22.0	176	June 84	2.2	0.16
	22.0	162		1.7	0.13
	22.4	164		1.5	0.11
	20.8	172		1.5	0.11
	22.8	226		1.2	0.09
	35.0	514		2.3	0.17
July 85	27.7	318	May 85	21.5	142
	27.2	290		20.5	112
	27.0	278		20.2	114
	25.0	232		20.0	112
	29.0	372		19.6	96
	38.0	796		22.0	164

alkalinity and calcium weekly to verify that water quality did not interfere with the experiment. Artificial tungsten lighting above each tank provided 25 lux during daylight hours. Fish were measured (cm T.L.) and weighed (g) at the beginning and the end of the experiments. Oocytes were sampled periodically by catheterization (Ross 1984) and preserved in 5% formalin for subsequent measurements and staging. Oocytes were measured under a stereomicroscope with a calibrated ocular micrometer. They were then examined for morphological maturation and were classified into four categories:

I. primary oocyte—white without yolk formation, 0.10–1.15 mm,
II. yolk vesicule stage—opaque yellow with yolk vesicules, 0.85–2.40 mm,
III. yolk globule stage—clear yellow with a yolk globule, 1.65–2.45 mm, and
IV. germinal vesicule stage—clear yellow with the migration of yolk globule and the apparition of a white spot-germinal vesicule, 1.95–2.55 mm.

Experiment I: Wild Broodstock

Smallmouth bass ranging from 20.8 to 35.0 cm T.L. and weighing 162–514 g (Table 1) were maintained in an 800 L rectangular tank throughout the experiment, October 1984 to June 1985. These fish were exposed to the following photoperiod and temperature:

a) constant long days (18L:6D) and warm temperature (24 C) for 6 weeks;
b) decreasing daylength (18L:6D to 8L:16D) and decreasing temperature (24 C to 6 C) over a 3 week period;
c) constant short days (8L:16D) and low temperature (6 C) for a 6 week period);
d) increasing daylength (8L:16D to 18L:6D) and increasing temperature (6 C to 24 C) over a 3 week period; and
e) constant long days (18L:6D) and warm temperature (24 C).

Experiment II: Laboratory-Reared Broodstock

Six virgin smallmouth bass, ranging from 19.6 to 22.0 cm T.L. and weighing 96–164 g (Table 1) were transferred to an 11,000 L round tank in April after being reared in an 800 L tank for 10 months, June 1984 to April 1985. The experiment terminated a month later, May 1985, with spawning. These fish were exposed to the following photoperiod and temperature regimes:

a) constant long days (18L:6D) and warm temperature (24 C) during the first 10 months of their life;
b) constant long days (18L:6D) and decreasing temperature (24 C to 10 C) over a 10 day period; and
c) constant long days (18L:6D) and increasing temperature (10 C to 24 C) over a 10 day period.

Results

Experiment I: Wild Broodstock

Wild smallmouth bass broodstock exposed to a compressed annual temperature and photoperiod cycle showed oocyte maturation but no final gonadal maturation and ovulation at the end of the cycle (Fig. 1). Oocytes, stage I, at the beginning of the experiment matured to stage II as water temperature and photoperiod decreased. A month later, when photoperiod became 18L:

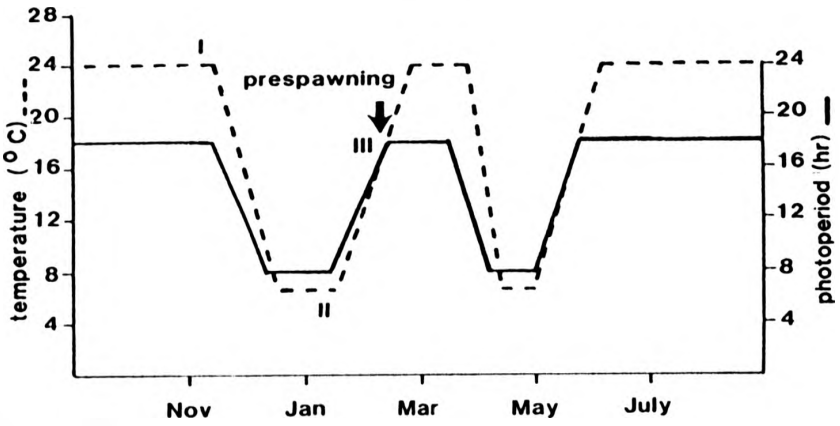

FIGURE 1. *Experiment I—Temperature and light regimes to control reproductive cycle of wild smallmouth bass. Roman numerals I–IV indicate morphological maturation categories of the oocytes as described in the text.*

6D and temperature was still increasing, oocytes reached stage III at the same time that prespawning behaviour was observed. This behaviour was characterized by territorial defense and nest building on the provided substrate and occurred when the temperature reached 19 C and the photoperiod 18L: 6D. For some reason, no further oocyte maturation was observed, and the fish did not spawn. A subsequent compressed photoperiod and temperature cycle over a month period resulted in oocyte regression.

Experiment II: Laboratory-Reared Broodstock

Laboratory-reared bass maintained under constant temperature and photoperiod regimes (Fig. 3) attained sexual maturity 10 months after hatching compared to 5–6 years for wild fish in Quebec (Dubois, personal communication). The reared fish spawned at 11 months at 20.5 cm T.L. Coble (1975) reported the smallest smallmouth bass to spawn as being 22.5 cm T.L. Oocytes, of fish hatched in June 1985, matured from stage I to II by January 1986 and remained constant until April, when a decrease in temperature to 10 C over 10 days at 18L: 6D constant photoperiod advanced oocyte maturity to stage III and elicited the prespawning behaviour. A subsequent temperature rise to 20 C over 10 days was associated with increased maturity to stage IV followed by spawning in May (Fig. 2).

Discussion

The arrest of oocyte maturation and subsequent oocyte regression in the wild broodstock smallmouth bass after displaying prespawning behaviour suggest that some variables other than photoperiod and temperature cycle may have inhibited spawning. Stacey (1984) pointed out that regulation of teleost ovulation is under control of both endogenous and exogenous factors. That females of many teleost species complete vitellogenesis but fail to ovulate in captivity indicates that activation of the ovulatory mechanism requires exogenous stimuli which are both distinct from those required for ovarian recrudescence and lacking in the artificial environment. In the two experiments, cyclic and constant regimes did not inhibit the endogenous rhythm in terms of oocyte maturation, and under a constant optimal regime, time to attain final stage of maturation was accelerated. So, why did the sexually mature fish in Experiment

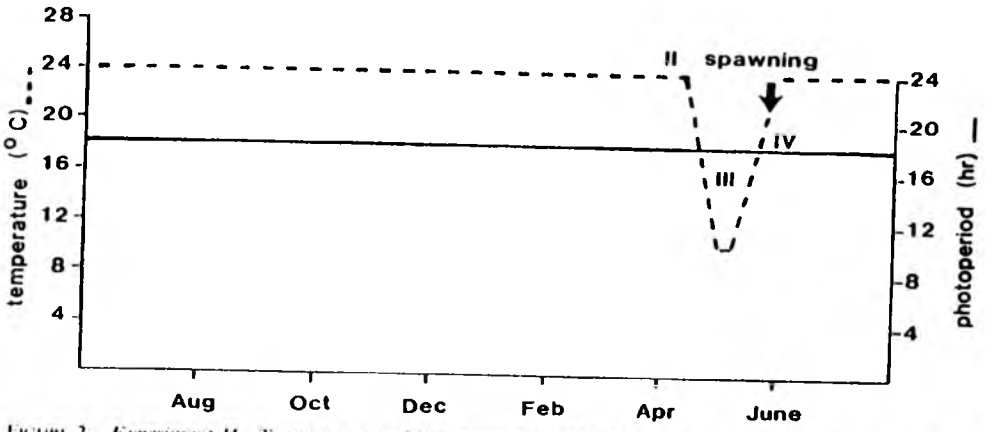

FIGURE 2. *Experiment II—Temperature and light regimes to induce first time spawning of laboratory-reared smallmouth bass. Roman numerals I–IV indicate morphological maturation categories of the oocytes as described in the text.*

I not spawn after displaying prespawning behaviour? Based on the fish behaviour, two critical factors that need to be looked at are duration of a long photoperiod cycle and vital space.

Scott et al. (1984) mentioned that a long photoperiod may be required for a specific duration to promote complete oocyte maturation. In Experiment I, the duration of a long day photoperiod was three months compared to ten months for Experiment II prior to being exposed to a particular temperature and photoperiod regime. The short duration of long photoperiod, for the wild broodstock, might have affected the completion of oocyte maturation. The minimal length of long day photoperiod (18L:6D) required to induce final oocyte maturation in smallmouth bass has yet to be defined. It is important for smallmouth bass, a territorial species, to have enough space between nests to minimize male agonistic behaviour such as observed in our experiments. The 800 L tank used for spawning may not have been adequate for two males. Vision plays an important role in their spawning activities. Vital space can probably interfere with the fragile synchronisation essential to spawning success in smallmouth bass. Gross (1980) pointed out that mating success for sunfish is influenced by the physical environment and the social interactions occurring at the nest site. The behavioural observations confirmed that while social interactions between male and male are agonistic, the male and female interactions are more of an independent type. Also, as observed in nature (Sura et al. 1985) the prespawning and spawning locations are the same for the males but different for the females. Thus, it seems essential for the females to have access to enough space not to inhibit their prespawning behaviour and allow final oocyte maturation to take place.

Results of Experiment II with laboratory-reared bass agree with recent findings of Bromage et al. (1984) and Scott et al. (1984) that constant long photoperiod can induce spawning, and also suggest that acute temperature change may have triggered spawning behaviour and accelerated oocyte maturation to the point of ovulation.

The similarity of early stages of oocyte development in both Experiments I and II, in spite of different environmental conditions, suggests that oocyte maturation can

occur independently of environmental factors although the rate may be affected (deVlaming 1975). Therefore, the possibility exists that early phases of gonadal development are controlled by an endogenous rhythm and that photoperiod and/or temperature modulate this development and serve as triggers for ovulation. Exogenous factors, such as vital space, could also play a decisive role in final gonadal maturation. The immediate spatial environment seems to be critical for male-female synchronisation and spawning of smallmouth bass.

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