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Dynamic aspects of ovogenesis in an asynchronous fish, the gudgeon *Gobio gobio* L. (Teleostei, Cyprinidae), under controlled temperature and photoperiod conditions

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Abstract

Dynamic aspects of an asynchronous cyprinid's ovogenesis, the gudgeon *Gobio gobio* L., were examined by a histo-morphometric approach and effects of two factors, temperature and photoperiod, were investigated in respect to the stage of maturation. Four stages of maturity (one protoplasmic or vacuole free oocyte, two vesicle stages, also called in endogenous vitellogenesis and one vitellogenic or in exogenous vitellogenesis) were identified and compared to macroscopic characteristics of maturity (gonadosomatic index and condition coefficients, K and K 1). Growth and relative proportion to the entire ovary of each oocyte stage was measured on the different experimental groups. Relative importance of temperature and photoperiod were clearly dependent on the gametogenic stage (recrudescence, final maturation and ovulation, and regression). Under constant temperature and photoperiod conditions, a strong vesicle stage oocyte regression was observed; a periode of low temperature or decreasing photoperiod seemed necessary to allow gonad recrudescence to be initiated. An annual cycle contracted into 6 months accelerated gametogenesis and produced off-season spawnings. Contraction of the reproductive cycle did not entail a reduction of oocyte growth or the yolky oocyte proportion. During the spawning period, females ovulated several times with a 15-day interval between successive spawnings. Vesicle and globule stages were induced by increasing temperature or photoperiod, but final maturation and ovulation were primarily dependent on temperature. The regressive stage was caused by a decreasing photoperiod and/or temperature. From a group synchronous distribution of oocytes at the start of recrudescence, the gudgeon's ovary changed to a totally asynchronous distribution at the end of vitellogenesis.

Keywords : *Gobio gobio*, gametogenesis, asynchronous ovary, temperature, photoperiod.

Aspects dynamiques de l'ovogenèse chez un poisson asynchrone, le goujon Gobio gobio *L. (Téléostéen, Cyprinidé) sous conditions contrôlées de température et de photopériode.*

Résumé

Par une approche histo-morphométrique, nous avons étudié la dynamique de l'ovogenèse chez un poisson cyprinidé à développement ovocytaire asynchrone, le goujon, *Gobio gobio* L. et déterminé l'influence des facteurs environnementaux proximaux (température et photopériode) selon le stade de maturité des poissons. Quatre stades de maturité (un protoplasmique, non vacuolisé, deux prévitellogéniques, également appelés en vitellogenèse endogène et un vitellogénique ou en vitollogenèse exogène) ont été déterminés et comparés aux critères macroscopiques, tels que le rapport gonadosomatique et les coefficients de conditions, K et K1. La croissance de chaque stade ovocytaire et sa proportion relative dans l'ovaire ont été déterminées dans les différents groupes expérimentaux. Il apparaît que l'importance relative de ces deux facteurs est étroitement dépendante du stade de maturité des gonades et varie selon la phase de recrudescence, de maturation finale et ovulation et de régression. Sous conditions constantes de température et photopériode dès le démarrage de la recrudescence gonadale,

nous observons une nette régression des ovocytes en prévitellogenèse. Au contraire, un cycle de température et/ou de photopériode contracté sur 6 mois accélère la gamétogenèse et permet l'obtention de pontes hors-saison. Cette contraction du cycle de reproduction n'entraîne toutefois pas de réduction dans la taille des ovocytes ou dans la proportion relative des ovocytes vitellogéniques dans l'ovaire. Durant leur période de reproduction respective, les femelles ont pondu à plusieurs reprises avec un intervalle de 15 jours entre chaque ponte. Les vitellogenèses endogène et exogène peuvent être induites par une température et/ou une photopériode croissante, mais la maturation finale et l'ovulation requièrent des conditions thermiques plus strictes. La phase de régression est provoquée par une température et/ou une photopériode décroissante. A partir d'une distribution groupe-synchrone des ovocytes en début de recrudescence, l'ovaire de goujon évolue progressivement vers une distribution totalement asynchrone en fin de vitellogenèse.

Mots-clés : *Gobio gobio*, gamétogenèse, ovaire asynchrone, température, photopériode.

INTRODUCTION

In species that only spawn during a short period of the year, the opportunity to obtain larvae is restricted to the few weeks during which the fish are viable. However, the adults must be maintained year-round in tanks or ponds. Knowledge of the factors involved in the gametogenetic process should benefit aquaculture by acceleration, maintenance or delay of a predetermined maturation stage, induction or inhibition of final maturation and ovulation, off-season spawning and increase of fry recruitment (Shehadeh, 1970; De Vlaming, 1974; Scott, 1979; Pullin and Kuo, 1981; Lam, 1983; Bye, 1984; Poncin, 1988).

It is generally assumed that temperature and photoperiod are the most important factors (Baker, 1938) functioning as advance signals of a suitable reproductive season (De Vlaming, 1974). From the literature, it appears that environmental influence varies depending on the stage of gametogenesis (gonad recrudescence, final maturation and ovulation, regression). De Vlaming (1972) clearly showed that short-term laboratory experiments can lead to erroneous conclusions about the importance of exogenous factors controlling annual reproductive cycles.

Previously (Kestemont, 1987), seasonal variation was described ovary histology for gudgeon, *Gobio gobio* Linnaeus 1758, held in a pond. This small european cyprinid, which is of increasing importance as a baitfish for sport fishing, develops oocytes asynchronously. In the present work dynamic aspects of gametogenesis in breeder animals reared in tanks were examined and the influence of controlled temperature and photoperiod was studied to identify stage of gonadal development by using a histo-morphometric approach to analyze oocyte growth and ovary maturation.

MATERIAL AND METHODS

Experimental facilities

This study was done in recirculating systems, each consisting of two 150 l rectangular tanks (2.74 × 0.45 × 0.23 m), a 200 l sedimentation tank and a 250 l biological filter. After filtration, water was well aerated and disinfected by UV radiation. Photoperiod was simulated using fluorescent lights producecing an illumination of 1 000 lux at the water's surface. The temperature for each group was regulated by a heater-pump with a precision of 0.5 °C. To simulate the winter temperature, a cooler-pump was inserted into the recirculation systems. Dissolved oxygen was maintained close to saturation. NH_4^+ and NO_2^- were checked weekly and were maintained, respectively, below 0.5 and 0.2 mg/l.

The fish were fed continuously during the light period by an automatic feeder. The daily ration of pellet feed (Trouvit 1) was maintained *ad libidum*.

Experiment 1: environmental induction of gonad recrudescence

In September 1986, five hundred 3-year old gudgeon were purchased from a local fish farm, divided into five groups and exposed to different combinations of temperature and photoperiod (*fig.* 1).

Group 1: Natural temperature and photoperiod conditions. Control group, held in a pond.

Group 2: Constant temperature (20 °C) and photoperiod (LD 12 : 12) (L = light, D = dark).

Group 3: Annual temperature and photoperiod cycle contracted into 6 months. The rate variation of these two parameters was twice as fast as the control.

Group 4: Annual photoperiod cycle contracted into 6 months at a constant temperature (20 °C).

Group 5: Annual temperature cycle contracted into 6 months with a constant photoperiod (LD 12 : 12).

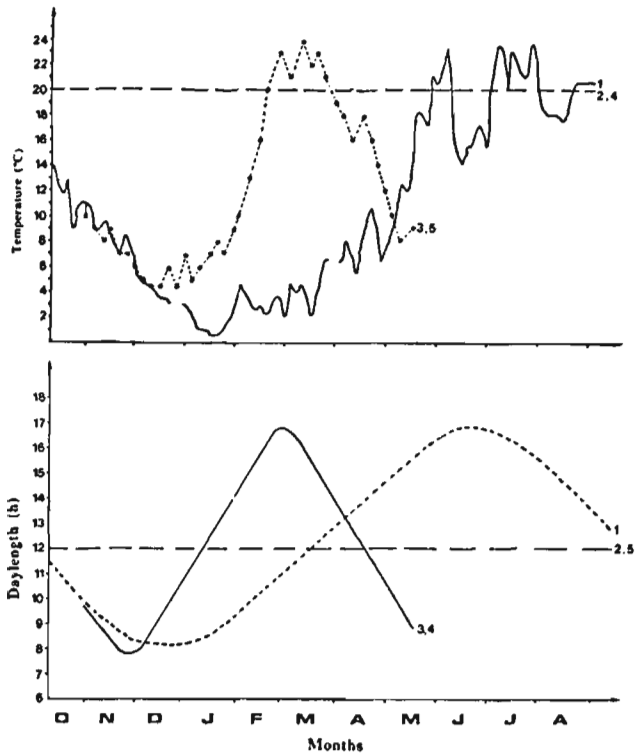

Figure 1. – Profiles of temperature and daylength in the different experimental groups of gudgeon *Gobio gobio* L.

Criteria of maturation

Fishes were checked weekly to observe the first signs of maturity, *i.e.* the presence of nuptial tubercles and spermiation in males and a swollen abdomen and ovulation in females. During the spawning season, a partial ablation of the pelvic fins allowed determination of the number of spawnings by each female. Each month five males and five females from each group were sacrified in order to histologically examine the stage of gametogenesis. In this paper, only female conditions are described.

Body weight and total lenght of the fishes were recorded and gonads were weighed and fixed in Bouin's solution. Ovaries were prepared into 6 mm sections and stained with trichrome Hemaluin, phloxine and light green (Langeron, 1942; Romeis, 1948). Ovary development was evaluated by the gonadosomatic index and by the coefficients of condition (Le Cren, 1951) as follows:

Gonadosomatic index (GSI) = $100 \times (W_0/W_f)$.

Coefficients of condition, $K = 100 \times (W_f/L^3)$ and $K_1 = 100 \times (W_f - W_0)/L^3$ where W_f = weight of the entire fish, W_0 = weight of the ovary and L = total lenght of the fish.

Histological analysis was done by determining the four stages of oocyte development as described by Kestemont (1987):

Stage 1: Protoplasmic stage or vacuole free oocyte: oogonia and primary oocytes have a vacuole-free cytoplasm, without yolk substance.

Stage 2: Vesicle stage or endogenous vitellogenesis: cortical alveoli with glycoprotein inclusions in two or three rings in the cytoplasm periphery.

Stage 3: Vesicle stage or endogenous vitelllogenesis: enlarged oocytes and cytoplasm filled with cortical alveoli from the zona radiata to the nucleus.

Stage 4: Globule stage or exogenous vitellogenesis: accumulation of lipoprotein vitellus in the cytoplasm. Central or peripheral germinal vesicle and appearance of micropyle.

The atretic or post-ovulatory follicles were only observed qualitatively. Any oocyte just starting a regressive phase was not taken into account.

Dynamic analysis of ovarian development

Ovary development was examined by a histo-morphometric approach similar to that described by Kestemont (1987). Two parameters were examined: 1) distribution of oocyte size, evaluated by measuring 100 profiles in each maturation stage, and 2) relative proportion of each stage, *i.e.* counting 1,000 cells per ovary and then dividing the percentage of a defined stage by the corresponding oocytes mean diameter. Quantitative analysis takes into account stereological corrections based on the wicksell transformation (Weibel, 1979). Two other parameters that influence size distribution were considered: correction coefficient for truncated profiles (Holmes effect) and effect of section thickness (Aherne and Dunhill, 1982).

Experiment 2: induction of vitelllogenesis

100 adult gudgeons were collected by draining a pond in mid-December 1986, then under a short photoperiod (LD 8:16) and at a low temperature (3-4 °C) and were then reared under experimental conditions as described above. Daylength was fixed at LD 12:12 and temperature was slowly increased to 15 °C and then maintained for 3 months (mid December-mid March). This temperature was slightly lower than the temperature required for gudgeon spawning. Indeed, several authors (Bernet, 1960; Brunet and Hoestlandt, 1972) indicated that a temperature of 16-17 °C is required to obtain a spontaneous spawning in gudgeon. In mid-March 1987, the temperature was raised to 20 °C. Maturation parameters were: external characteristics (checked weekly), GSI and microscopic observations as described previously (checked monthly).

Experiment 3: induction of final maturation and ovulation

Breeders used in this experiment were reared in a pond during most of their reproductive cycle. Gudgeons were collected from the pond in mid-April 1987

and divided into four groups of 20 fish. After 4 days of acclimatization in the tanks, the temperature was raised to the required level.

Group 1: constant 20 °C and natural daylength.

Group 2: constant 15 °C and natural daylength.

Group 3: constant 20 °C and LL photoperiod.

Group 4: constant 20 °C and DD photoperiod.

One month later (May 15), five females from each group were sacrificed. Parameters examined were GSI and microscopic observation of the ovaries histology.

Data analysis

Data from the GSI, K and K1, and mean diameter of the oocytes were analyzed at each stage by a two-way variance (ANOVA 2) (Dagnelie, 1975). Size of the most advanced oocytes for the different groups was also examined. An Hartley test was carried out to verify the homogeneity of variance. It was shown in a previous paper (Kestemont, 1987) that one section of gonad and one field per section are representative of the whole ovary.

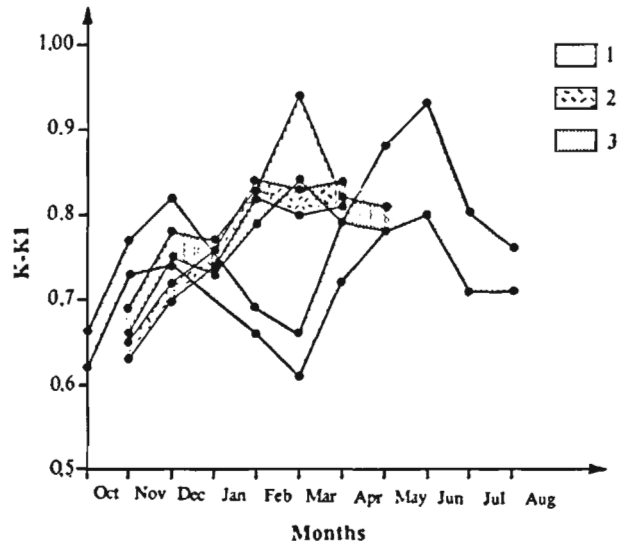

Figure 2. – Variations of the coefficients K (upper line) in control group (1), constant conditions group (2) and condensed conditions groups (3). The variation of K and K 1 in groups 4 and 5 were similar to that of group 3.

Results

Effects of external factors on gonad recrudescence

The study was conducted from October to August for the control group and from October to May for the experimental groups. Gudgeon tolerate well captivity as well as the articial food (Trouvit 1, Trow & Co, Belgium). However, during the spawning season, we observed a set of spontaneous ovulations but no oviposition. Ova resorption was critical and sometimes induced an internal haemorrhage causeing a bloody abdomen in the ovulated females. Checking the fishes regularly together with manual evacuation of ova by applying abdominal pressure usually prevented development of further problems.

Coefficients of condition and gonadosomatic index

The coefficients of condition showed a variation which clearly corresponded to the variation of temperature and photoperiod (*fig.* 2). Group two's coefficients of condition (constant 20 °C and LD 12:12) did not vary compared to the control group. The increase of K was caused by growth of the fishes rather then by development of gonadal mass. The difference between K-K 1 did not increase significantly during the experiment.

The GSI was also significantly lower than in the other groups (3, 4 and 5) except in the control group which underwent hibernation conditions (*fig.* 3). In groups 3, 4 and 5 reared under contracted cycles of temperature and/or photoperiod, the coefficients K and K 1 showed a variation pattern condensed into 6 months. The increase of K (and the area K-K 1) was highly significant ($p<0.001$) and was directly related to these two factors. In contrast, there was no significant difference between the changes of these three groups.

The GSI variation was also highly significant ($p<0.001$). The end of active spawning and the decrease of GSI corresponded to daylenght decrease. The same phenomenon was observed with decrease of

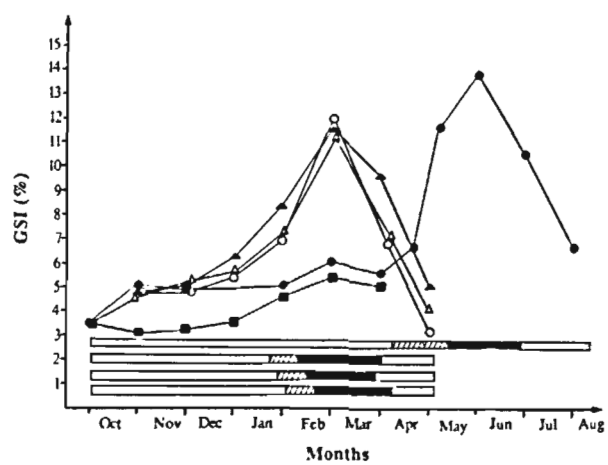

Figure 3. – Changes in the gonadosomatic index of gudgeon held under different combinations of temperature and photoperiod. Group 1, ●; 2, ■; 3, ○; 4, ▲; 5, △; (⊥), standard deviation. Horizontal lines indicate the presence of gravid females (////) and ovulated females (▬).

Table 1. – Sum of degree-days (real and efficient $\theta > 10$ °C) before first spawning in different groups of experiment 1.

Group	Date of first spawning	End of maturity	Degree-days	efficient degree-days
1	19/5	end of July	1,498	113
2	–	–	–	–
3	15/2	3-10/04	942	119
4	18/2	3-10/04	2,346	966
5	22/2	15-25/04	1,062	179

temperature, independenty of photoperiod. No significant difference in the GSI of groups 3, 4 and 5 appeared during the experiment although the GSI of group 4 (constant 20 °C) was slightly higher from October to February. The difference between group 4 and groups 3 and 5 was reduced when temperature increased (*fig.* 3). During active spawning, some females ovulated three times with two week intervals between successive ovulations. In the control group, the first signs of female maturation occurred in May (*fig.* 3). *Table* 1 indicates the dates and sum of degree-days before the first ovulation. However, below 8 to 10 °C, there was no vitellogenic accumulation and the sum of effective degree-days was then calculated with $\theta > 10$ °C. It appears that a high temperature under short daylength (group 4) did not accelerate maturation and thus the sum of effective degree-days was higher (966 in group 4; 119 in group 3; and 179 in group 5).

Histological examination of gonad development

– *Control* (*group* 1): Ovary development of gudgeon held in a pond was described in detail in a previous paper (Kestemont, 1987). In October, all fishes were at the same stage of development and only contained stage 1 and 2 oocytes. These stages were present through the entire annual cycle in percentages of 66 to 85% for the stage 1 and 6.5 to 16% for the second stage. Stage 3, reached in November, continued until April as the most advanced stage (*fig.* 4). Very low temperature blocked gonad maturation at this stage during the winter and early spring. In May, an acceleration in gonad development and the appearance of yolky oocytes were observed. Enlargement of the oocytes(up to 1,100 µm diameter) by accumulation of york globules caused a marked increase in GSI. This stage was only present from May to July and the ovary then started a recovery phase and only contained stage 1 and 2 oocytes. During this period, temperature was high but photoperiod was decreasing.

– *Constant 20 °C and LD 12:12* (*group* 2)*:* After 1 month of rearing, fishes showed variable gonad development. Some fishes matured normally, although slowly, with an accumulation of yolk vesicles, while some others started a regressive phase. This regression mainly occurred in vesicle stage oocytes (stages 2 and 3). During the following months, this phenomenon intensified and some ovaries were in complete regression. In ovaries showing normal development, the oocytes and their yolk vesicles were smaller than in the control group. The yolk globule stage was never reached and in February we observed oocytes only at stages 1 and 2. In March and April, stage 3 was again present but the largest oocytes were generally atretic.

– *Condensed temperature and photoperiod* (*group* 3)*:* During the first months of the experiment (October to December) this group changed like the control. The lenghtened photoperiod in December, intensified in January by the increase in temperature, induced acceleration of the recrudescence phase. In February, one female with yolky oocytes was noted. Only a few atretic follicles were observed during active spawning. In April, some fishes spawned again while some others were in a recovery stage. Yolky oocytes disappeared by May, females were either in a recovery stage or in recrudescence, with stage 2 and 3 oocytes.

– *Constant 20 °C and condensed photoperiod* (*group* 4)*:* Gonad development of this group was similar to the one observed in the previous group (3). Only a slight advance of gametogenesis (also shown by GSI) was noted during the first months, probably caused by the higher water temperature. Nevertheless, the accumulation of yolk gobules (stage 4) was not observed until February. Yolky oocytes, central or peripheral germinal vesicles, and post-ovulatory follicles were observed in the sample from March and April. However decreased daylength, initiated in March corresponded to the end of spawning and regression of many oocytes in April.

– *Condensed temperature and constant LD 12:12* (*group* 5)*:* In this group, ovary development appeared to be regulated only by temperature. Endogenous and exogenous vitellogenesis were similar to those of groups 3 and 4. However, the active spawning stage was longer than the two other groups. Indeed, a suitable temperature (higher than 16-17 °C) was maintained until the second half of April and several ovulations occurred again during the second half of this month.

Dynamic analysis of oocyte growth

Effects of exogenous factors appeared at different stages of ovogenesis. It acted on the recruitment processes or on the rate of oocyte growth. Also it could influence the relative proportion of each oocyte stage in the ovary. To understand oocyte growth in an asynchronous ovary, the fact that ovogenesis is a continuous process must be taken into account. In a given stage, the growth of oocytes increases the mean size of that stage, but growth of the largest oocytes into the next stage (e.g. by inclusion of yolk vesicles or yolk globules) or the entry of small oocytes from

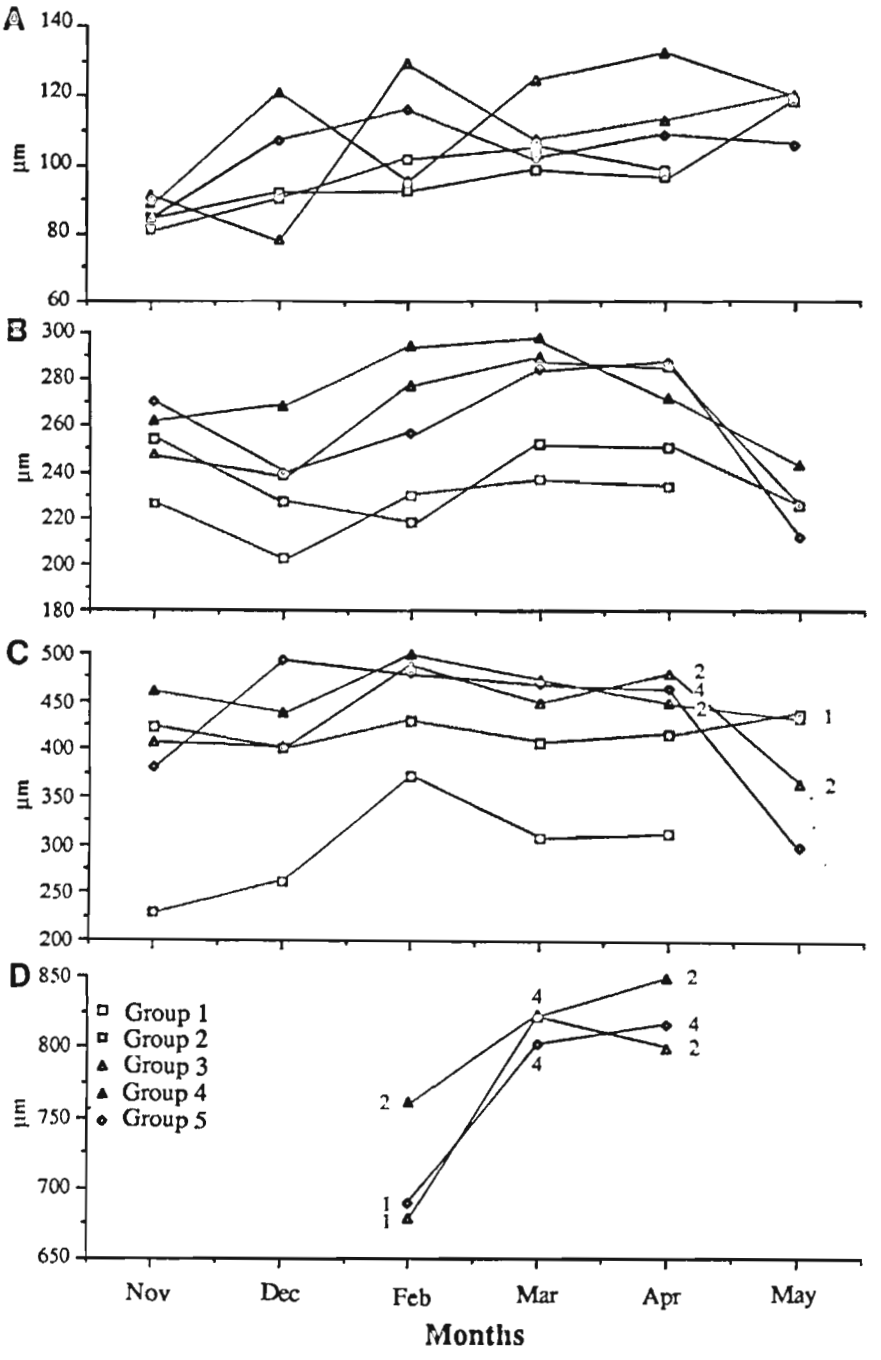

Figure 4. – Change in mean size (diameter) of oocytes in the different groups (1-5). 1 = protoplasmic stage; 2 = start of endogenous vitellogenesis; 3 = end of endogenous vitellogenesis; 4 = exogenous vitellogenesis. When the stage was not present in all fishes, the number of fishes studied is noted.

the lower stage produces a decrease in the mean diameter of this stage.

o *Variation in mean size of oocytes*

Stage 1: Except in the control group which showed no significant variation from November to April, the variation in size of protoplasmic oocytes was significant in the other groups (*fig.* 4*a*). In group 2, recruitment of small oocytes occurred normally although mean size of stage 1 increased slowly, from 81 µm in November to 105 µm in March. In the three other groups, oocytes diameter changed markedly with time.

Generally, under decreasing temperature and/or photoperiod (from November to December), there was no recruitment of protoplasmic oocytes and mean

size also decreased because the largest oocytes progressed to endogenous vitellogenesis. However, in the fourth group, recruitment started earlier, probably because of the high temperature. From December to February, increase of temperature and daylength in groups 3 and 5 produced growth of oocytes. During active spawning, recruitment ceased and mean size of protoplasmic oocytes decreased. The ANOVA 2 indicated a significant effect of treatment and time of sampling on size of oocytes at stage 1.

Stage 2: Except for oocyte size of group 2, which was similar to or lower than those of group 1, mean sizes of the other groups were significantly higher up to April (*fig.* 4b). Under constant temperature and photoperiod, pre-ovulatory regression was considerable and mainly affected the most advanced oocytes; this explains the low size of stage 2 (202 to 237 µm). At the start of the experiment, a slight decrease of mean size was observed in groups 3 and 5, caused by recruitement of small protoplasmic oocytes in endogenous vitellogenesis. This recruitment declined in February and mean size of vesicle stage oocytes increased progressively until the reproductive period (300 µm). In group 4, a faster growth of oocytes was noted in November, probably promoted by the high temperature 20°C. At the end of spawning, the important regression of oocytes produced a sharp decrease in mean size.

Stage 3: The profile of stage 3 oocyte growth was similar in the different groups, except in group 2 where it was clearly low (*fig.* 4c). The Hartley test of variance homogeneity did not allow a variance analysis to be done. During the simulated spawning season (March and April), mean size of oocytes in the condensed cycles (groups 3, 4 and 5) was slightly lower than those of group 1 during the corresponding period (June and July). In April, stage 3 was again present in four females of group 5 and only in two females of groups 3 and 4. Stage 3 oocytes grew again during spawning, since mean size of this stage remained constant despite of the fact that many large oocytes entered into vitellogenesis.

Stage 4: This stage was present in groups 3, 4 and 5 during February, March and April. It appeared in May only in group 1. However, not all of the females contained vitellogenic oocytes during these three months. The number of fishes studied in each group are shown in *figure* 4d. Oocyte size reached in the contracted profiles were similar to those of group 1 during the normal reproductive season (Kestemont, 1987). A reduction of thermal and/or photoperiodic

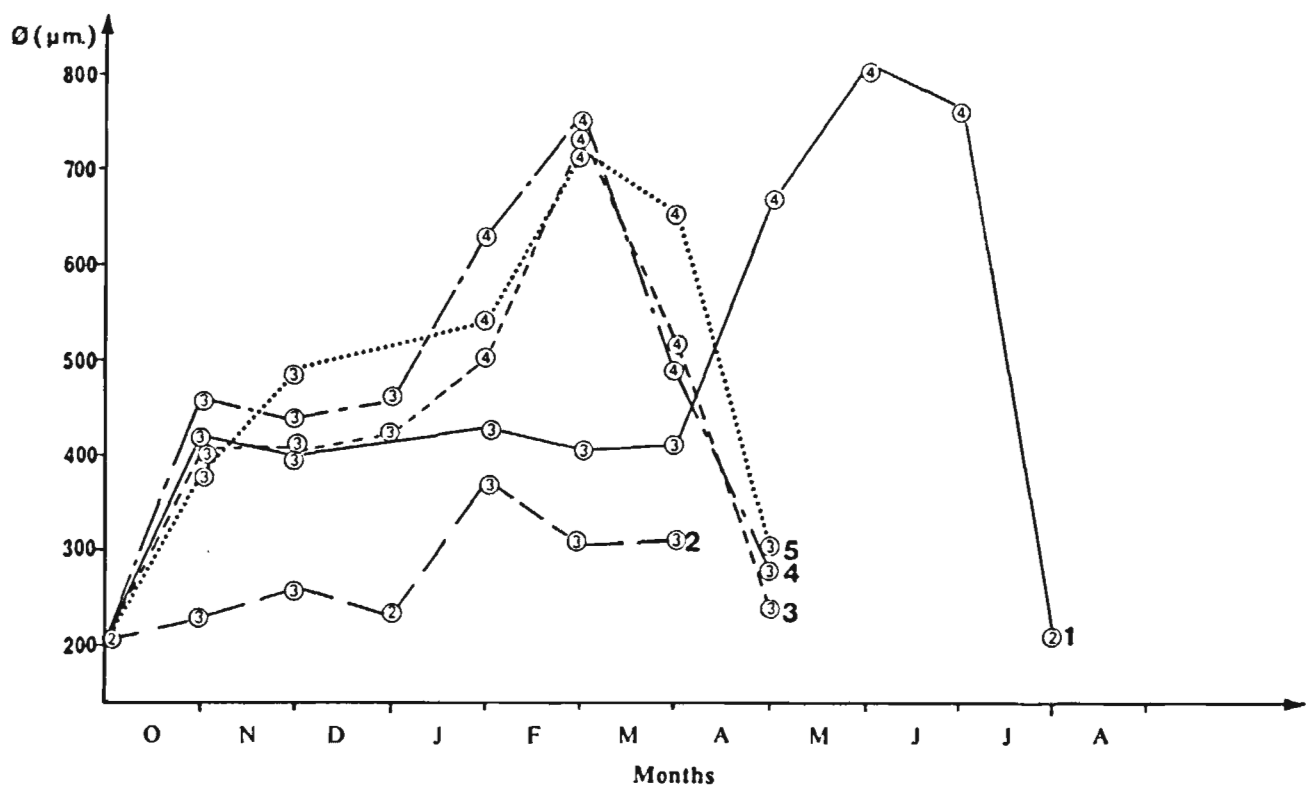

Figure 5. – Change of stage and mean diameter of the most advanced oocytes in the different groups of gudgeon. Group 1 ——, 2 – –, 3 —, 4-.-., 5.... .

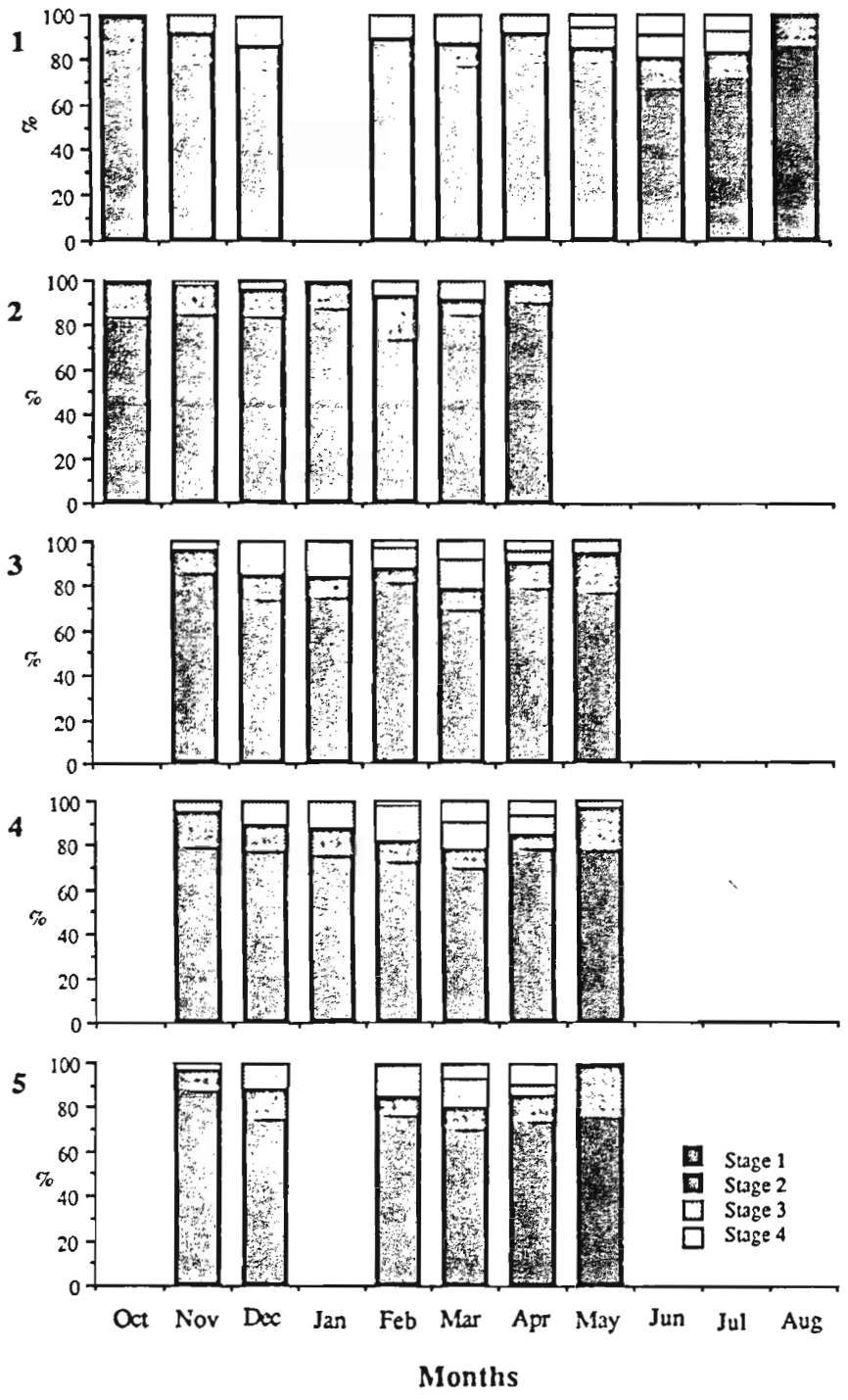

Figure 6. – Percentage of each oocyte stage in gudgeon of the different experimental groups (1 to 5).

cycles did not entail a reduction of yolky oocyte growth.

Most advanced stages in each ovary: The previous analysis of each stage only considered fishes which showed a definite stage. Therefore, these values represent mean oocyte size at a certain period, but not the maturity of the whole population during this period. Indeed, some values were representative of only one or two ovaries. Growth of the most advanced stage is shown in *figure* 5. All sampled fishes were included; thus mean size of a mentioned stage may differ from the indicated value.

• *Proportion of each oocyte stage*

In all groups, protoplasmic oocytes were largely predominant (*fig.* 6). Their presence was only reduced during the respective spawning periods. Experimental conditions mainly acted on recruitment and growth of vesicle and globule stage oocytes. The increased percentage of yolky oocytes observed in March in groups 3 and 4, and in April for group 5 was similar to that of group 1 in June (*fig.* 7), and was not reduced by contraction of the sexual cycle.

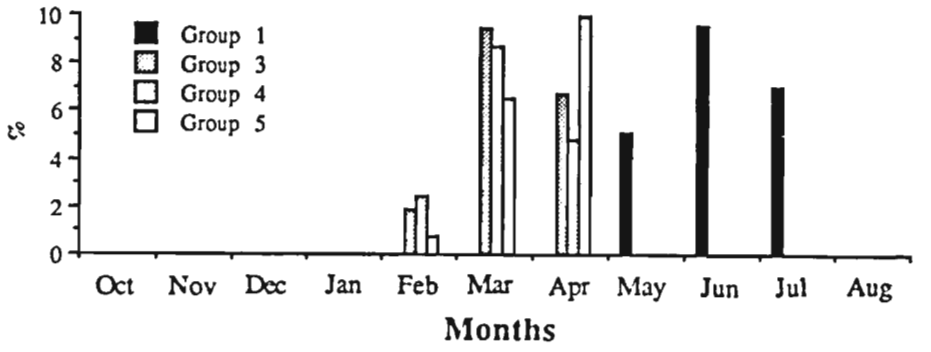

Figure 7. - Comparison of yolky oocyte percentage in ovary of the gudgeon, *Gobio gobio* L., in each experimental group during their respective spawning season.

Induction of vitellogenesis

Fishes sampled from the pond in mid-December were in the same maturity stage as fishes of group 1. Endogenous vitellogenesis appeared to be completed in many of the oocytes (end of stage 3). In February, several females had a swollen abdomen and the GSI was clearly higher than in group 1 (*table* 2). Most of the ovaries contained yolky oocytes and several were completely filled with yolk globules. A similar situation occurred in March, wherein exogenous vitellogenesis was completed but final maturation did not occur (migration and breakdown of germinal vesicles were not observed) and ovulation did not occur. In contrast, increase of temperature from 15 to 20 °C in mid-March induced several ovulations over the next days.

Table 2. – Gonadosomatic index (GSI) and relative abundance of each maturity stage in ovaries of the gudgeon, *Gobio gobio* L. maintained on LD 12:12 photoperiod and 15 °C from mid-December to mid-March. ( ) = standard deviation; + rare, + + common, + + + abundant.

Maturation stage	Period of samplings		
	December	February	March
Protoplasmic oocytes	+ + +	+ + +	+ + +
Start of endogenous vitellogenesis	+ +	+	+
End of endogenous vitellogenesis	+ + +	+ +	+ +
Exogenous vitellogenesis	–	+ +	+ +
GSI	3.5 (0.4)	9.7 (1.3)	10.8 (1.8)

Induction of final maturation and ovulation

Fishes pond sampled in April did not tolerate experimental conditions very well, probably due to the closeness of spawning which made the fishes more delicate. Several fishes were infected by ectoparasites (*Trichodina* sp. and *Ichthyophtirius* sp.) generally associated with bacterial infections (*Pseudomonas* sp.). Examinations were carried out only on healthy fishes.

GSI was at a maximum in group 1 (16.5 ± 1.6) and at a minimum in group 2 (8.5 ± 1.8) (*table* 3). At the start of the experiment, all females contained small yolky oocytes. One month later, maturation had progressed slightly in group 2 where vitellogenesis was completed but ovulation did not occur. Histological observations did not show any postovulatory follicle or micropyle and all germinal vesicles were in the central position again. In group 1, several females spawned and contained post-ovulatory follicles. Oocytes at the final maturation stage were also observed under constant light (group 3) but ovulation did not occur and the GSI was significantly lower than in group 1. Under constant darkness, vitellogenesis and final maturation occurred normally and some females ovulated spontaneously. However, oviposition was not observed.

DISCUSSION

Like other species from temperature regions (for a review see De Vlaming, 1974; Lam, 1983; Bye, 1984), the reproductive cycle of gudgeon, *Gobio gobio* is highly dependent on temperature and photoperiod. This fact was previously stressed by different authors (Bernet, 1960; Penaz and Prokes 1978; Kestemont, 1987). This study shows the relative importance of these two factors on the dynamics of ovarian development.

Influence on gonad recrudescence

Available data in the literature indicate that in cyprinids, temperature is the most important factor

Table 3. – Gonadosomatic index (GSI), ovulation and oviposition of gudgeon female, *Gobio gobio* L., maintained under different combinations of temperature and photoperiod from the beginning to end of April. L = light, D = dark; ( ) = standard deviation.

Group	Combination T°/photoperiod	Initial GSI (%)	Final GSI (%)	Ovulation	Oviposition
1	20/Natural	6.0 (0.7)	16.5 (1.6)		+
2	15/Natural	6.1 (1.1)	8.5 (1.8)	–	–
3	20/LL	5.8 (0.5)	10.8 (2.6)	–	–
4	20/DD	6.2 (1.0)	12.4 (1.4)	+	–

involved in determining the sexual cycle, although few experiments have been carried out to demonstrate the role of photoperiod (Billard, *et al.*, 1978; Scott, 1979; Davies and Hanyu, 1986; Poncin, 1988). In carp *Cyprinus carpio*, the next gametogenetic cycle commences immediately in spent fish, and may be completed as early as October. Development is then suspended until the environment is warm enough for final maturation and ovulation (Bye, 1984).

In gudgeon, gonad recrudescence starts in early autumn, after one or two months of quiescence following summer spawning, but is blocked at the third oocyte stage (end of previtellogenesis) during winter (Kestemont, 1987). A similar change was observed in the experimental groups. Decreasing photoperiod allowed the start of gametogenesis but the rate of gonad recrudescence was increased by high temperature (20 °C). Indeed, fishes of group 4 showed a slight advance in size and proportion of vesicle stage oocytes in December and January. In groups 3 and 5, low temperature induced a slower accumulation of glycoprotein vesicles and thus a slower oocyte growth rate. Recruitment of protoplasmic oocytes in endogenous vitellogenesis was also less important at the start of the cycle. In January, increase of temperature and/or photoperiod corresponded to accumulation of yolk vesicles and growth of stage 3 oocytes. In *Fundulus confluentus*, the first stage of oocyte growth occurs at low temperature and the following stages at higher temperature (Harrington, 1959). In contrast, endogenous vitellogenesis in *Cymatogaster aggregata* requires high temperature and exogenous vitellogenesis low temperature (Wiebe, 1968). In *Notemigonus crysoleucas* (De Vlaming, 1975) and *Gasterosteus aculeatus* (Baggerman, 1980), endogenous vitellogenesis does not depend on environmental factors, although the rate of development can be affected. In gudgeon, accumulation of yolk globules required high temperature and a long photoperiod (at least 12 hours of light). Indeed, this process appeared at the same time and in relatively similar proportions in three groups (3, 4 and 5). The only difference was the size of yolky oocytes. Rearing gudgeons at high temperatures (20 °C) over several months did not cause stage 4 to be reached since the photoperiod was short or decreasing. Similarly, increasing photoperiod did not induce the vitellogenic process at an inhibiting temperature. Thus, it appears that endogenous vitellogenesis occurred over a wide range of temperatures and photoperiods, but that exogenous vitellogenesis required stricter conditions. At constant temperature (20 °C) and daylength (LD 12:12), gonad recrudescence was greatly inhibited. It seems that variation of environmental factors was very important to allow gonad development. The responsiveness to these two exogenous factors was clearly dependent on gonad receptivity which is itself a function of maturation (effects vary with season) De Vlaming, 1974. Examples were previously reported in different species of teleosts (Gillet *et al.*, 1978; Baggerman, 1980; Lam, 1983; Bye, 1984). In gudgeon it is probable that constant temperature (20 °C) and photoperiod (LD 12:12) could positively influence gonad maturation after the fishes had completed endogenous vitellogenesis and recruitment of small oocytes.

Influence on final maturation and ovulation

From the results of experiment 3, it appears that temperature was the main factor inducing final maturation and ovulation, and that photoperiod only played a secondary role. Indeed, although endogenous mechanisms must be in operation, functional maturity was attained at high temperature 20 °C under continuous light or total darkness. Furthermore, rearing gudgeons since December under constant photoperiod LD 12:12 and temperature (15 °C) (experiment 2) provided mature females with yolk oocytes after February but ovulations were only observed when temperature increased to 20 °C. This major effect of temperature on ovulation has been reported in many species of cyprinids (Kossman, 1975; Gillet *et al.*, 1978; Breton *et al.*, 1980; Horoszewicz, 1981; Poncin *et al.*, 1987). However, recent work on maturation and spawning of carp, *Cyprinus carpio* (Davies and Hanyu, 1986; Davies *et al.*, 1986 *a*, b) also stress the role of photoperiod.

Environmental induction of gonad regression

In gudgeon, the regressive phase was coordinated by temperature and/or photoperiod. Indeed, a decreasing photoperiod at a suitable temperature (group 4) progressively induced atresia of vesicle and globule stage oocytes. The same phenomenon was observed in group 3 where the combination of these two factors caused a sudden regression of mature oocytes. Females of group 5 remained mature until

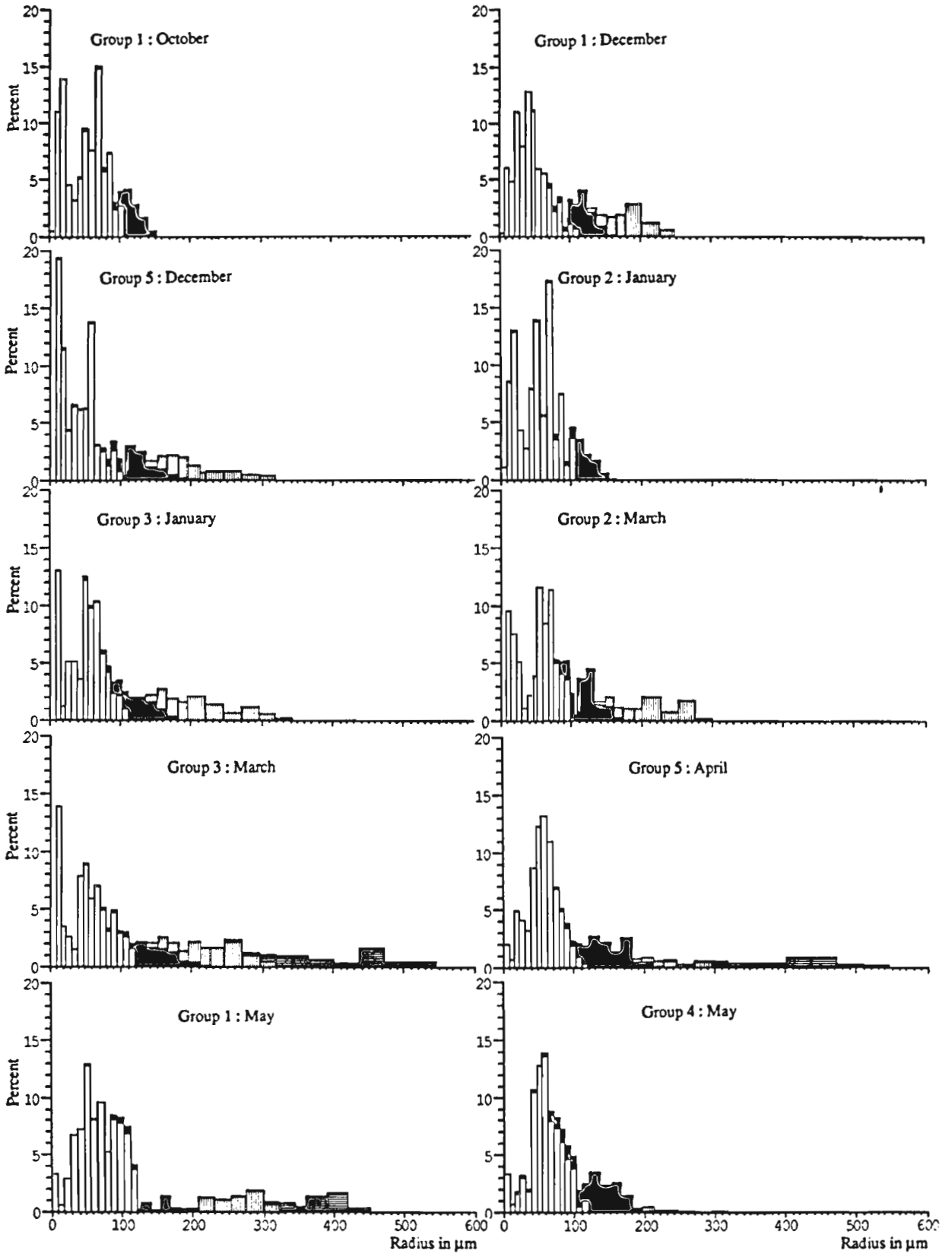

Figure 8. – Distribution of oocyte size in the ovary of gudgeon *Gobio gobio* L. during the most representative sampling periods in the different groups.

mid-April (constant photoperiod and suitable temperature) and then regressed when temperature decreased. A similar phenomenon was mentioned by De Vlaming and Paquette (1977) which indicated that high temperature associated with short photoperiod caused atresia but prevented this regression at the end of spawning when combined with a long photoperiod. Poncin *et al.* (1987) also reported regression induced by a decreasing photoperiod in *Barbus barbus*, *Tinca tinca* and probably *Leuciscus cephalus*, even when temperature was suitable.

An endogenous rhythm of post-spawning regression had been suggested for several teleosts (see reviewed by Lam, 1983) based on the fact that suitable environmental conditions unavoidably lead to a "refractory stage" after spawning. Peter (1981) suggested that ovarian regression in goldfish, *Carassius auratus*, was related to the lack of a daily cycle of serum gonadotropin level, even if the GTH level was high. It is possible that a sustained gonadotropin secretion caused constant stimulation of gonadotropin receptors in the ovary leading to inactivation.

Another hypothesis can be proposed on the basis of our dynamics analysis. Indeed, we observed a cessation of recruitment of protoplasmic oocytes during yolk globule accumulation and active spawning (natural or simulated). Because during recrudescence there was a continuous maturation of oocytes followed by a set of repeated spawnings (at 15-day intervals) and a lack of stage 1 oocyte recruitment, females became progressively deprived of mature oocytes and failed to spawn. In barbel, Poncin (1988) observed during one spawning period as many as 15 successive spawnings followed by a refractory period when females were "exhausted". Furthermore, Wallace and Selman (1981) emphasized that protoplasmic growth was independent of GTH concentration. So, the high levels of serum gonadotropin probably present during spawning were ineffective to induce recruitment of stage 1 oocytes.

Effects of environmental factors on oocyte recruitment

Recruitment, shown in *figure* 4, was variable according to oocyte stage and experimental group. In group 2, constant conditions of temperature and photoperiod allowed a normal recruitment of stage 1 and stage 2 oocytes.However, the growth rate of stage 2 oocytes was lower than in the other groups. Stage 3 oocytes were rare and mostly in atresia. Measurements of pituitary GTH concentrations by ratio-immunoassay (unpublished data) showed that the GTH levels had strongly decreased in fishes of group 2 during the first months of the experiment. Since accumulation of yolk vesicles and yolk globules are GTH dependent (Wallace and Selman, 1981; Idler, 1982), the lack of hormonal stimulation inhibited normal development of the oocytes and their complete maturation.

In the contracted cycles, oocyte recruitment appeared similar to that of fishes held in the pond, although clearly faster. In February, size of stage 4 oocytes and their relative proportion in the ovaries of the three groups were comparable to those of group 1 during the normal spawning season. The phenomenon of repeated spawnings, reported under natural conditions (Bernet, 1960; Mann, 1980; Mann *et al.*, 1984; Kestemont, 1987), was maintained under artificial surroundings. Stripping each egg represented about 10% of body weight. Thus, acceleration of gametogenesis does not seem to reduce fecundity of gudgeon as it was observed in tench, *T. tinca* (Breton *et al.*, 1980; Epler *et al.*, 1989) when temperature was quickly increased.

According to Marza (1938) who defined three patterns of oogenesis (synchronous, group-synchronous or asynchronous), we can classify the ovaries of gudgeon kept under natural or artificial conditions at the limit of the two latter patterns. Indeed, *figure* 8 clearly indicates that, from a group-synchronous distribution of oocytes at the start of gametogenesis (with a prevailing class of protoplasmic oocytes), the ovary of the gudgeon changed progressively to a totally asynchronous distribution at the end of vitellogenesis.

It is important to note that this work was carried out on a wild stock of gudgeons reared for two years under natural conditions in pond. Further studies are thus necessary to confirm these results with a captive fishes reared under artificial conditions since hatching. Some parameters like fecundity, quality of eggs and larvae must be considered to evaluate the real advantage of this kind of control on intensive culture of gudgeon.

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