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Twice-annually spawning rainbow trout females in a cultured population from southern Chile. I. Biometric performance and reproductive cycle dynamic.

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Abstract

Two rainbow trout female populations (Wt-01 y Wt-02) were studied, which a percentage of individuals displayed a double annually reproductive cycle (DARC). This trait is characterized by an autumn spawning (normal reproductive cycle) and an additional one at spring (additional reproductive cycle). During seasons 2004 and 2005, the specimens that registered an additional spawning besides the normal, included 14.4% (Wt-01) and 36.2% (Wt-02) of the population. These females belong to double-cycler specimens, while those who spawn once a year are mono-cyclers. In both populations, the reproductive performance of normal reproductive cycle was comparatively evaluated in relation to the additional reproductive cycle, using seven biometric-reproductive variables. The average weight of the spawning females (Wt-01: 1139 ± 116 g vs 1528 ± 105 g; Wt-02: 1195 ± 217 vs 1512 ± 176), the total fecundity (Wt-01: 2.310 ± 214 vs 3388 ± 299; Wt-02: 2.691 ± 600 vs 3.680 ± 509) and the relative fecundity (Wt-01: 2.051 ± 278 vs 2.226 ± 299; Wt-02: 2271 ± 420 vs 2461 ± 354), exhibited significant differences in favour of the additional reproductive cycle ($p < 0.05$). Nevertheless, the eggs diameter (Wt-01: 5.03 ± 0.09 mm vs. 4.70 ± 0.18 mm; Wt-02 : 4.89 ± 0.16 mm vs 4.58 ± 0.23 mm), the fertility rate (Wt-01: 86.6 ± 15.0 vs. 80.8 ± 11.9; Wt-02: 94.5 ± 7.5 vs 77.8 ± 24.9) and the eyed eggs stage survival rate (Wt-01: 83.89 % ± 7.70 vs 77.62 % ± 9.45; Wt-02: 84.73 % ± 11.80 vs 68.84 % ± 15.95), were significantly

higher in the normal reproductive cycle. The quality of the eggs obtained during the additional cycle, was acceptable and compatible with it productive utilization. Analysis of additional reproductive cycle length (RCLa), the period time from autumn spawning date to spring spawning date, increases according to the spring spawning season progresses. First spawning females Wt-01 and Wt-02 had a RCLa average of 134 days (30/09/04) and 149 days (14/10/05) respectively, while the last spawners had a RCLa average of 187 days (29/11/04) and 217 days (03/02/06), respectively. In the same way, an eggs diameter increment was observed in relation with the spawning date progress during the additional reproductive cycle in Wt-01 as well as in Wt-02, showing high correlation values ($r = 0,973$ y $r = 0,850$, respectively). Additionally, the normal reproductive cycle length (RCLn) studied in a third population (Wt-03), shows a decrease tendency in relation to the progresses of the date of spawning in previous spring spawning. The RCL dynamic of normal and additional reproductive cycles in double-cycler females could be associated to an energetic compensation that supports the high cost for a female to ovulate twice in a year. A greater dispersion or asynchrony is observed in the spawning date distribution over the additional cycle in regard to the normal cycle, which could be a consequence of the heterogeneity observed in RLC.

Introduction

The salmonids reproduction is characterized for being an annual event in which the gonadal growth that begins in spring, gives place to spawning that will be carried out in the next months of autumn and winter (Duston and Bromage, 1987). In rainbow trout, for example, the histological changes of the ovary are visible a year before the mature oocytes are ovulated (Elliot et al 1984; Sumpter et al 1984).

Rainbow trout reproduction, and probably of all salmonids, is initiated and subsequently modulated by photoperiodic cues derivative from the seasonally-changing cycle of daylength, (Whitehead *et al*, 1978; Bromage *et al*, 1982[a]), having temperature only a secondary role (Henderson 1963; de Vlaming, 1972). The photoperiod, more than initiating and modulating directly the reproductive cycle in rainbow trout, would be used to activate a biological clock that, in short, controls the gonadal maturation and spawning (Duston and Bromage, 1987;

Bromage and Cumaranatunga, 1988). This control is expressed in a physiological level, by means of the different hormone and neurotransmitter secretion patterns of the hypothalamus–hypophysis–gonad axis (Billard et al, 1978; Whitehead *et al*, 1978; Bromage *et al*, 1982a ; Elliot et al 1984).

Reproduction is a physiological process that demands a high energetic cost. For this reason, in natural populations, events like sexual maturation and spawning have evolved to coincide with optimal environmental, climatic and food availability conditions. These allow taking optimal advantage of the resources by the progenitor and its progeny (Leder et al. 2006). In salmonids farming, that fitting is irrelevant, and in contrary, considerable advantages exist achieving reproduction out of season and ideally, the permanent production of eggs from a breeding stock (all-year-round or out of season eggs*)* (Bromage and Duston, 1986). The principal efforts oriented to achieve this goal have been carried out by the means of photoperiodic manipulations over spawning timing, which is relatively simple in salmonids (Bromage and Duston, 1986). Nevertheless, the spawning date dispersion, the decrease in the eggs size and the failure of reaching over a 50% of response to those treatments, have impeded the massive diffusion of that technology specially in medium size or small farms (Bromage and Cumaranatunga, 1988). In this sense, it has been proposed that the use of strains which spawn every 6 months without photoperiodic control would be much more effective (Bromage and Cumaranatunga, 1988).

Even though, most biologists will consider it as an unusual behaviour, rainbow trout is able of spawning twice a year without photoperiodic manipulation (Gall and Crandell, 1992). This phenomena is determined by a double anually reproductive cycle (DARC). Nevertheless, the quality of the generated eggs by these stocks and the capacity to maintain the periodicity of a six months cycle under different environmental conditions, has not been proved yet (Bromage and Cumaranatunga, 1988). Rainbow trout specimens that show the DARC trait, have been reported in USA, Japan and Canada. They were first observed by Hume in the United States in 1955 (Hume,1955), later in Japan by Matsung in 1969 (Matsung, 1969), Kato in 1971 (Kato, 1976) and Ohto in 1973 (Ohto et al, 1977) and more recently in Rainbow Springs Hatchery (Thamesford,

Ontario), where at least one twice-spawner specimen was registered (Fishback et al. 2000). The only data published for a double-cycler rainbow trout strain maintained and selected several times in the same place, has been those reported by Otho et al. in 1977, in Kumagaya Branch from the Experimental Fishing Prefecture Station of Saitama. Further studies reported diverse reproductive biometric and endocrinological aspects of this strain (Aida et al,1984; Lou,1984; Lou et al 1984; Lou et al, 1986; Tazaki et al, 1993, Takano et al, 1995), however more recent data about the reproductive performance of this strain are unavailable.

In Chile, in the rainbow trout breeding center "Ojos del Caburgua" located in Pucón, we have found some populations where a fraction of the specimens display the DARC trait. We have observed that in the additional reproductive cycle expressed in these fish, normal and healthy embryonated eggs are produced. In the present study, various reproductive performance parameters and related aspects about their maturation timings of our twice spawners, are analyzed and discussed. It is of our interest to study in depth this character to understand better its reproductive biology, and promote its use in the farming eggs production.

Materials and Methods

The present study was developed between the years 2004 and 2007 in the rainbow trout breeding center "Ojos del Caburgua" owned by Piscícola Huililco Ltda., located in Pucón, Chile (S 39°14'29.5'' W 71°50'09.8'' y 280 meters over the sea level). Two all female populations that displayed DARC trait were used, which were born the years 2001 (Wt-01) and 2002 (Wt-02). This stocks were originated from the cross between females from the Wytheville strain (Wt), native from West Virginia USA, first imported to Chile early in the 80's, with neomales specimens, of Cofradex strain, whose stock was imported to Chile from Denmark in 1991.

Three-year-old specimens were evaluated during their normal and additional reproductive cycles corresponding to their first maturation. A third all female DARC population born in 2003 (Wt-03) was used to chronologically analyze the

second normal reproductive cycle when they were 4 years old, in relation to the previous additional cycle.

Fish were maintained in raceway using spring water with a temperature of 10 ± 1 ºC, with a water flow of 8-12 l/s and a culture density of 30 kg/m^{-3}. Food used was 5 mm extruded pellet (47% / 20% protein/lipid rate), with a feeding rate of 0.7% live weight/day. The reproductive management used (spawning, fertilization and incubation) was previously described (Estay et al. 1994; 1997; 1999). Tags were used on the dorsal fin to individually mark the females for 2004 season, while in season 2005, electronic tags were used (passive integrated transponders, TROVAN®), inoculated in the coelomic cavity during the first spawning.

In female populations Wt-01 and Wt-02, the values of seven biometric–reproductive variables were determined : spawning weight, fertilization rate, total and relative fecundity, eggs diameter, eyed eggs per female and eyed eggs survival rate. The total fecundity considered the total number of eggs produced per female, and the relative fecundity considered the number of eggs per every female kilogram. The fertilization rate considered the rate of viable embryos in a 10 eggs sample for every female, treated with a 30% acetic acid solution at 70 UTA. The eggs diameter average was estimated at the well pigmented eyed eggs stage (200 UTA) using a 300 mm ruler. The eyed eggs survival rate was estimated at 200 UTA. The reproductive cycle length (RCL) defined as the time interval (measured in days) from spawning date to spawning date of one female. The spawning period length (SPL, measured in days), was estimated for a cycle, using some females with individually spawning date identified.

Previous to the statistical analysis for every variable in each population, their normal distribution was proved using Kolmogorov-Smirnov test (Zar, 1974). For each population, variables were compared between normal and additional cycles using Student t test for independent samples (Zar, 1974) or otherwise a non parametric Mann-Whitney test (Siegel, 1957). Additionally, regression analysis were carried out to determine: a) the existing association between the progress of the spawning period and the eggs diameter in Wt-01 and Wt-02 populations, b) the existing association between RCL and the progress of the

spawning period, and c) the existing association between RCL and spawning date.

Results

1.- Spawning frequency

Population spawning distributions during normal and additional cycle are shown in Figure 1, for Wt-01 and Wt-02 populations. During reproductive season 2004 we detected DARC trait for the first time in Wt-01 population. Between March and July of that year, 988 Wt-01 spawning females were registered during their normal spawning period in autumn. Later, during spring of that same year, 142 of these same females (14.4 %) experienced an additional spawning (Figure 1a). During 2005 reproductive season, 995 Wt-02 spawning females were registered during their normal spawning period in autumn. Next spring, 360 of those same females (36.2 %) experienced a second spawning (Figure 1b).

2.- Biometric reproductive variables

The weight of spawning females and the main reproductive performance parameters of both populations are summarized in Table 1. In Figure 2, the weight interval distribution of females from population Wt-02 during normal autumn spawning in 2005 season is shown. A normal distribution is observed ($P > 0.05$), showing an average of 1150 g. The weight average comparation between mono-cycler vs double-cycler females, confirmed the absence of statistically significant differences for this variable (1146 ± 301 g vs 1175 ± 310 g, $P > 005$).

The reproductive productivity of both populations, comparatively evaluated in both cycles during their respective seasons through total fecundity averages and relative fecundity averages (Table 1), was in every case significantly higher during the additional cycle ($P < 0.05$). Nevertheless, the reproductive productivity, evaluated as for eggs average diameter, always resulted in significantly higher values during normal cycle ($P < 0.05$) (Table 1). As spring

spawning season progressed, an increasing tendency of the average diameter values of eggs was observed (Figure 3) in populations Wt-01 during 2004 and Wt-02 during 2005. In both cases, the determination coefficient (R^2) was high (Wt-01, R^2= 0,946; Wt-02, R^2= 0,722), which allows us to infer that at least 70 % of the eggs diameter variation is explained only by the progress of the spawning season.

On the other hand, the eggs viability evaluated through fertilization rate averages and eyed eggs survival rate averages, was in all cases significantly higher during normal cycle ($P < 0.05$) (Table 1).

3.- Temporal dynamic of DCRA phenomena

Some females of the populations Wt-01 (n= 38), Wt-02 (n = 258) y Wt-03 (n = 68) that maintained their individual tag during two consecutive spawning, allowed us to individually evaluate the dynamic of DARC phenomena through time. In these females, we were able to measure the length in days of the additional reproductive cycle (RCLa). When evaluating the average values of RCLa in Wt-01 during season 2004 and Wt-02 during season 2005, we could observe that, as spawning period progresses, RCLa values increased as it is observed in figures 4a and 4b. In both populations, the advance of the spawning season explains more than 90 % of RLCa variation. During season 2004, the first spawning Wt-01 females (30/09/04), registered a RCLa average of 134 days, while the last females in spawning (29/11/04), registered an RCLa average of 187 days. During season 2005 (Wt-02) a similar pattern was observed, the first spawning females (14/10/05), registered a RCLa average of 149 days while the last females in spawning (03/02/06) showed a RCLa average of 217 days. Regression curves between spawning date and RCLa, are shown in Figure 5a and 5b, presenting both seasons a positive and significant association ($P < 0.01$), with high correlation coefficients (r = 0.9031 and r = 0.9006, respectively).

When evaluating average length of the normal reproductive cycle (RCLn) on autumn 2007, using as reference those Wt-03 female groups with the same spawning date, during their previous additional spawning season 2006,

(Figure 4c), was observed an inverse pattern respect to that registered for RCLa during additional cycle (Figure 4a and 4b). That is to say, females that share more advanced spawning dates during previous additional cycle, show decreasing average values of RCLn respect to next normal cycle (Figure 4c). Again the progress of the spawning season explains more than 90 % of RCLn variation. The first Wt-03 females that spawned in spring (15/09/06), registered a RCLn average of 254 days, while the last to spawn (05/01/07), registered a RCLn average of 187 days. Figure 5c shows the corresponding regression curve between spawning date and normal RCLn, expressing a significant negative correlation ($r = - 0.8258$).

The spawning period length (SPL) was accurately measured using in each season, a sample of females whose individual spawning date during two consecutive spawns was known. In Figures 6a and 6b, it is possible to observe the SPL during normal and additional cycles of populations Wt-01 (7 days vs 60 days, n = 38) and Wt-02 (74 days vs 112 days, n = 258). In both populations, SPL for the same females is significantly more extensive in additional cycle, that is to say, the maturation process occurs with more synchrony during the normal autumn cycle.

Discussion

A rainbow trout strain capable of having two reproductive cycles in one year is a very unusual and very interesting reproductive biological phenomena, that also represents a huge productive economical potential. On one hand, it optimizes the eggs production process efficiency in a hatchery, because double-cycler specimens duplicate their production regarding a normal strain. On the other hand, it generates availability of embryonated eggs in an opposite season (spring), period when they normally scarce more.

Aida et al.(1984), propose that the DARC trait observed in the rainbow trout strain studied in the Experimental Fishing Prefecture Station of Saitama (Japan) would be under genetic control, since successive generations of its population maintained their twice annual spawning conduct, even when they were kept under natural photoperiod and different water temperatures. These authors reported a reproductive study about a 94-female population of this double cycler Japanese strain, and observed a winter spawning (normal cycle) between

November and January, involving 95.2 % of the females, and a summer spawning (additional cycle) between April and July, involving 62.3 % of the females. In our study, two populations of 988 and 995 females that registered their normal autumn cycle during years 2004 and 2005, respectively, exhibited an additional reproductive cycle during spring in lower frequencies (14.4% y 36.2%, respectively) than those described by Aida et al. (1984). It is possible that our fish with additional spawning show these differences in frequency because the studied populations in this work have not been previously selected for this trait.

Considering that this trait affects negatively the individuals' fitness, it is expected that DARC, in non selected populations, will be maintained in low frequency due to natural selection (Falconer y MacKay, 1996). Currently, we have preliminary evidences that indicate that the viability of double-cycler females is lower than mono-cycler females, which would support this hypothesis. On the other hand, it is interesting to notice that spawning dates have bimodal distribution (Figure 1), which would indicate the possible presence of a mayor gene or QTL that would be segregating in the population (Lynch y Walsh, 1988); this hypothesis is being tested in a backcross mapping panel, which result will be published in an independent work.

Rainbow trout native stocks spawning period in USA during IX century, as well as those stocks introduced more than a hundred years ago in Japan, was originally in spring (Gall and Crandell,1992; Lou et al,1986). This character, nevertheless, was systematically selectively pressured to obtain earlier spawns. For example, the average spawning time for rainbow trout strain from Hatchery Hot Creek (California, USA) changed dramatically since March in 1880, until October in 1940 and until August in 1980 (Lewis, 1944; Gall and Crandell,1992). This repeated selection of precocious spawns as long as water temperature over 10 ºC, would favor the early beginning of gonadal post-spawning recrudecence and then the expression of the DARC trait (Lou et al,1986). Coincidently, this temperature condition (over 10 ºC), also occur in Experimental Fishing Prefecture Station of Saitama as well as in our breeding center "Ojos del Caburgua" (10.6-13.5 ºC and 10.0 ±1 ºC respectively). Kincaid (1980) includes sexual maturation age as one of the qualities that distinguish rainbow trout strains. This author indicates that a close correlation exists

between this character and growth rate, and therefore a positive correlation between fast growth and early puberty.

Unlike Aida et al (1984), who showed and advantage in weight average for double-cyclers regarding mono-cyclers at the beginning of their season, we did not find in Wt-02 double-cycler females, analyzed during normal autumn spawning of season 2005 (about 6 months before additional spring spawning), significant differences in their weight average compared with mono-cycler sisters. So, it seems that females weight would not be determinant in DARC character expression.

When analyzing the reproductive productivity of the studied populations through total and relative fecundities, our result show that in both variables, the additional spring reproductive cycle presents significantly higher fecundity values. This contrasts the results obtained by Aida et al., (1984), who reported total fecundity averages of 4520 ± 215 eggs vs 6068 ± 219 eggs for 30 females, during their additional and normal cycles, respectively. Nevertheless, these authors in contrary to our study, evaluated the additional summer cycle before the normal winter cycle, that is, the inverse chronological situation in the life history of their fish, which could explain this difference. When considering our reproductive productivity results, it seems coherent that total and relative fecundity averages resulted superior during additional cycle due to the smaller eggs observed during this cycle (Table 1). This result is coherent with the inverse relation or "trade off" that is observed between these variables previously described by Springate and Bromage (1984b) in rainbow trout.

The eggs diameter average values always resulted lower during the additional cycle respect to normal cycle in the studied populations (Table 1). These results are consistent with the additional and normal cycle averages (3.94 ± 0.06 mm vs 4.24 ± 0.03 mm, respectively) reported by Aida et al. (1984) in their studied population. Also the progressive increase in the eggs diameter average observed in our double-cycler females during the additional spring cycle in both populations (Wt-01 and Wt-02), is consistent with Aida et al. (1984) observations, who during the additional summer cycle of their double-cycler females found eggs with smaller diameter in earlier spawning females, and bigger diameter eggs in later spawning females (3.55 ± 0.06 mm at the

beginning and 4.20 ± 0.20 mm at the end of the season). This phenomena could be correlated with the increasing RCLa observed in double-cycler females from both seasons (Wt-01 and Wt-02), as the additional spawning period progressed. Apparently, this RCLa determines a minor available lapse for vitellogenesis in early double-cycler females, producing a decreased yolk storage in the oocytes, which is the main responsible for eggs growth.

While comparing the reproductive performance through reproductive viability variable averages, like fertilization rate and eyed eggs survival rate, it was observed that, although double cycler females produce an acceptable performance during their additional reproductive spring cycle, in every case these values were significantly inferior comparing to the ones obtained during normal reproductive autumn cycle. These variables were not evaluated by Aida et al., (1984), neither by other authors, but could be related in some way with the smaller eggs size that females ovulate during additional reproductive cycle, especially in early spawning females.

Our results show that, as previously mentioned, double-cycler females present a progressively longer RCLa as spawning period progresses in the season (Figure 4). On the other hand, normal autumn reproductive cycle length (RCLn), at least in one population, showed an inverse dynamic. We postulate that this behavior could outline an energy compensatory mechanism, where early spawning females during additional reproductive cycle will afterwards be late spawning females in their next normal autumn cycle, and vice versa.

Another aspect that calls our attention in the population behavior of double-cycler females is the difference in synchrony that they show in spawning distribution when comparing SPL of the same females during their normal and additional reproductive cycles.

 SPL measurements carried out in subpopulation females with individual registration for every date in both populations studied show a higher spawning spring date dispersion in respect to autumn spawning. This is consistent with Aida et al, (1984) results. We believe that this asynchrony would be related to the heterogeneity observed in RCL from females who develop additional cycle.

For a female to exhibit DARC, and with that a RLC value of about 6 months for both cycles, vitellogenesis must start soon after its normal autumn spawning,

minimizing its reproductive resting period. Lou et al. (1984) detected as a critical period a month after normal spawning, in which mono or double-cycler condition is defined according to estradiol and Gth levels detected in plasma. These authors speculate that the growing spring photoperiod could delay summer spawning during additional cycle. Nevertheless, this does not explain the LCR differences found between early and late double-cycler females evaluated in this study who practically spawn in a 100 % during spring, but with a LCR of around 5 months in early females and of 6 to 7 months in late females. More work and analysis is yet needed to deepen into the observations reported in this study, especially studies that refer to ovary histology, hormone profiles and vitellogenin analysis that we are undertaking to report in a next study.

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Acknowledgments

Table 1. Reproductive biometric values in two rainbow trout populations
          carrying DARC character.

	Wt-01 season 2004		Wt-02 season 2005	
	Normal Cycle (n = 988)	Additional Cycle (n = 142)	Normal Cycle (n = 934)	Additional Cycle (n = 321)
	Average ± SD	Average ± SD	Average ± SD	Average ± SD
Female weight (g)	1,139 ±116 [a]	1,528 ± 105 [b]	1,195 ± 217 [a]	1,512 ±176 [b]
Fertilization rate (%)	86.6 ±15.0 [a]	80.8 ± 11.9 [b]	94.5 ± 7.5 [a]	77.8 ± 24.9 [b]
Total fecundity (Nº egg female)	2,310 ± 214 [a]	3,388 ± 299 [b]	2,691 ± 600 [a]	3,680 ± 509 [b]
Relative fecundity (Nº egg/Kg female)	2,051 ± 278 [a]	2,226 ± 299 [b]	2,271 ± 420 [a]	2,461 ± 354 [b]
Eggs diameter (mm)	5.03 ± 0.09 [a]	4.70 ± 0.18 [b]	4.89 ± 0.16 [a]	4.58 ± 0.23 [b]
Nº eyed eggs per female	1,920 ± 256 [a]	2,716 ± 77.6 [b]	2,341 ± 616 [a]	2,608 ± 678 [b]
Eyed eggs survival rate (%)	83.89 ± 7.70 [a]	77.62 ± 9.45 [b]	84.73 ±11.80 [a]	68.84 ± 15.95 [b]

Different letters in columns indicate significant statistical difference (P<0.05)
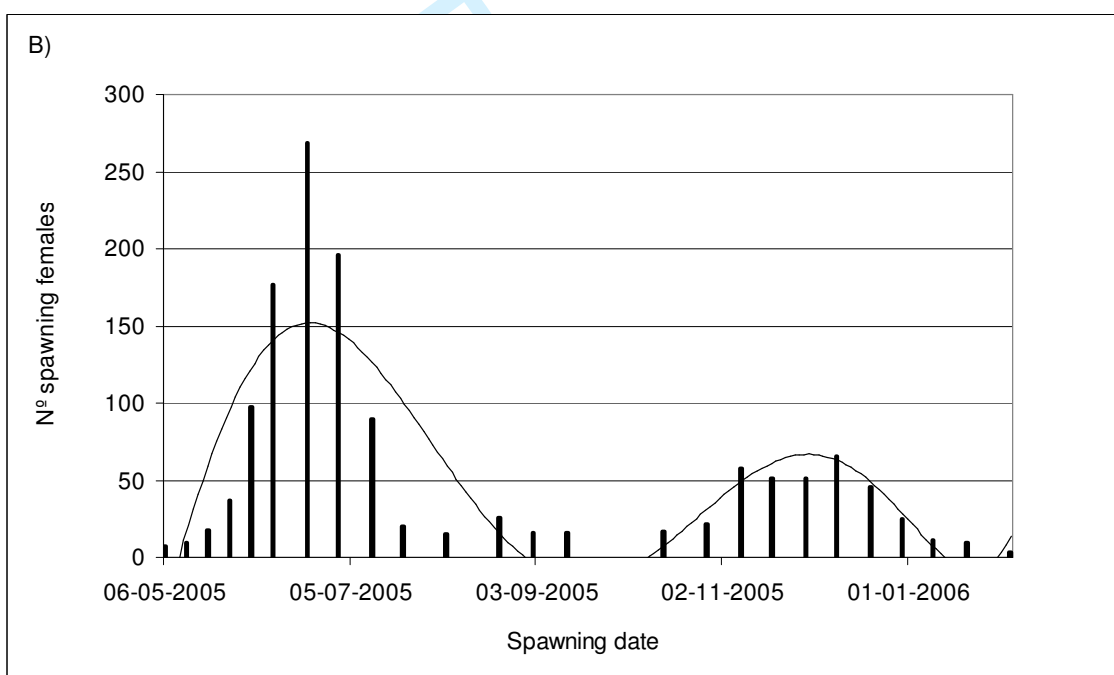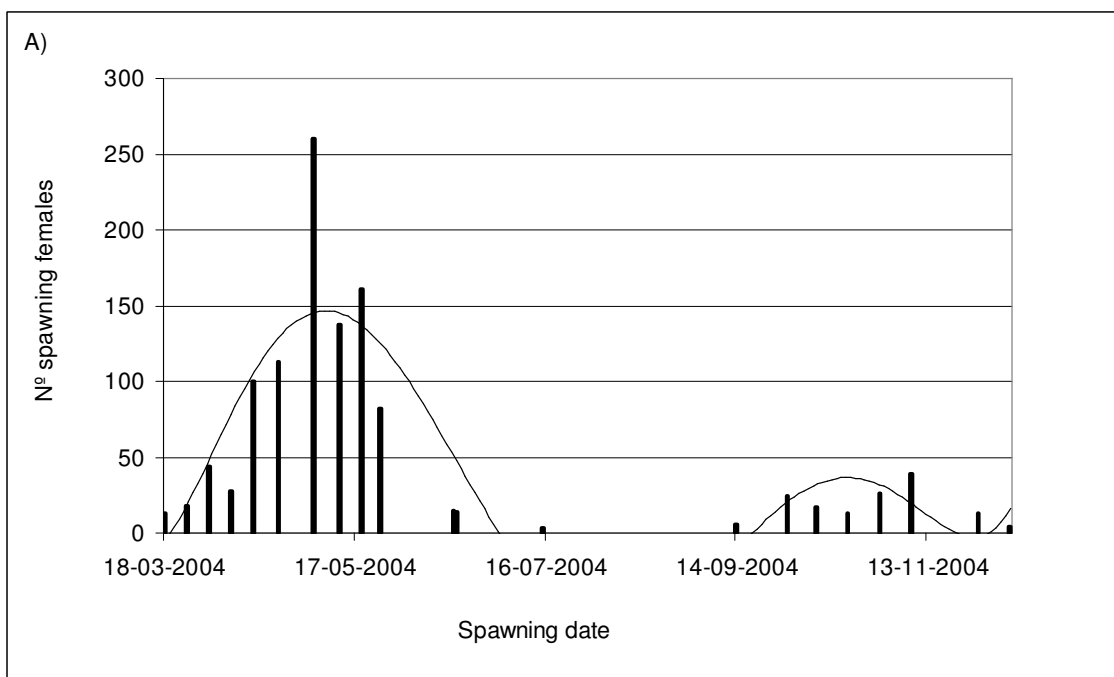
Figure 1. Normal and additional spawning date distribution in two female rainbow trout populations with double annually reproductive cycle trait.

a) Wt-01 population, season 2004   b) Wt-02 population, season 2005.
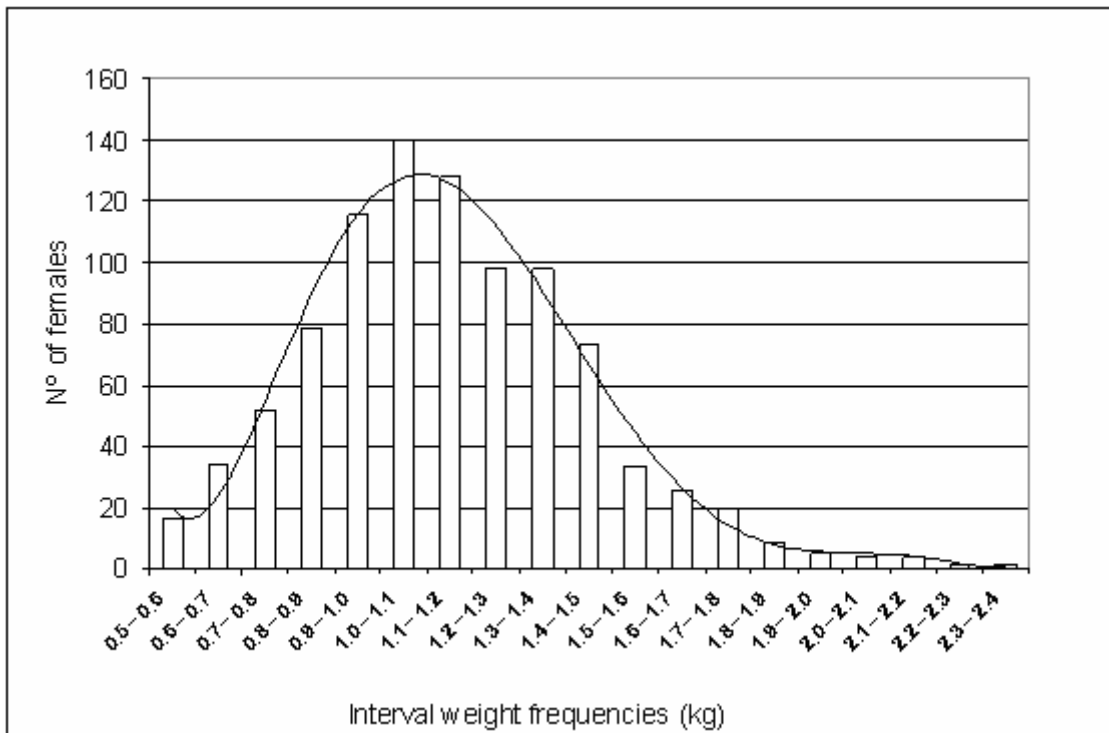
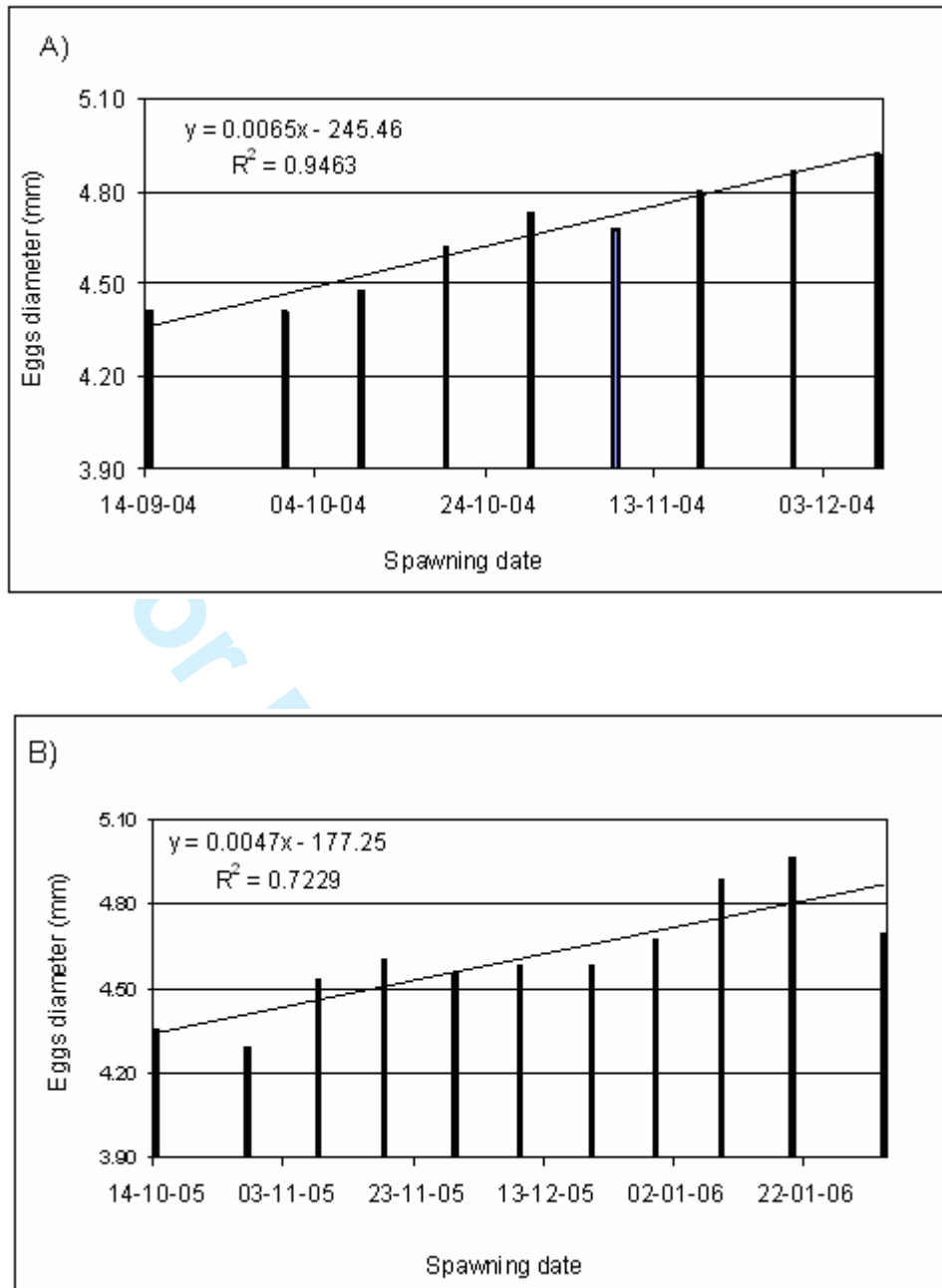Figure 2. Interval weight frequencies in Wt-02 females during normal cycle, 2005 spawning season.

Figure 3. Eggs diameter average evolution during spawning period progress in additional cycle. a) Wt-01 population, 2004 spawning season, b) Wt-02 population, 2005 spawning season.
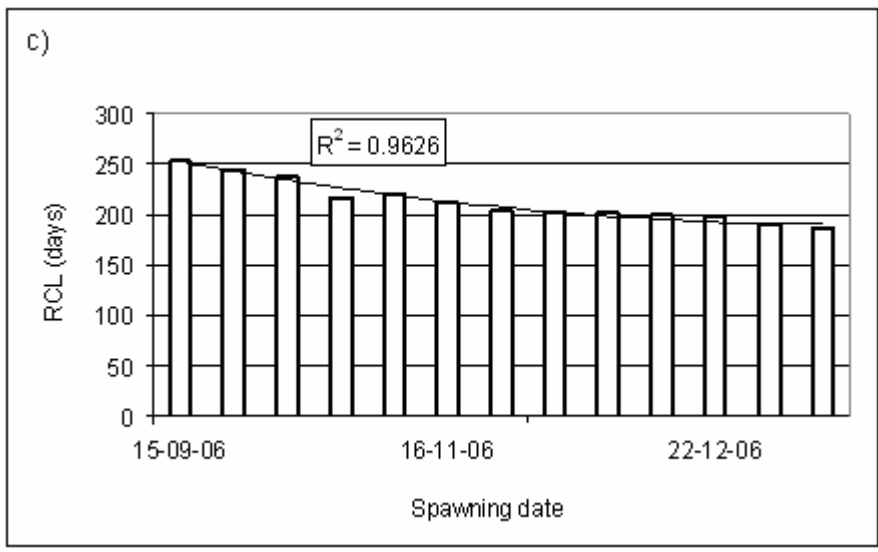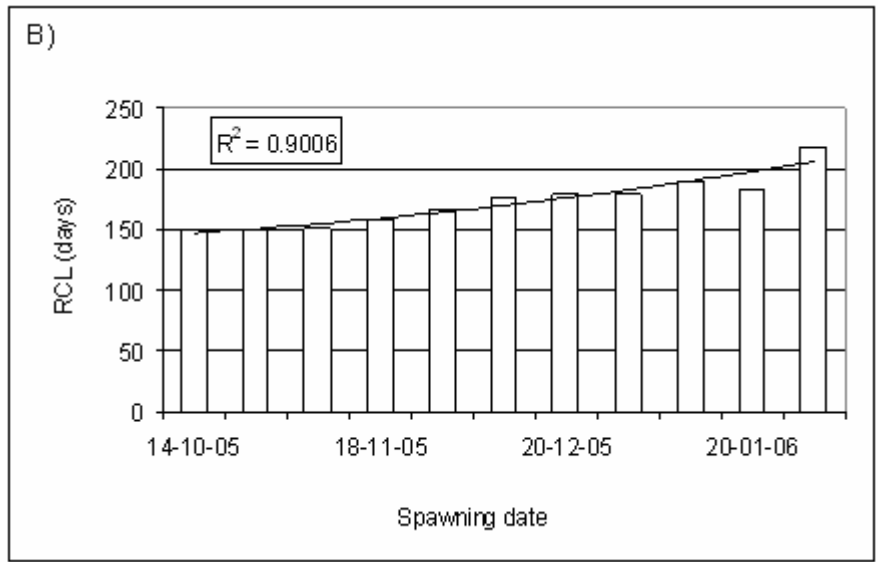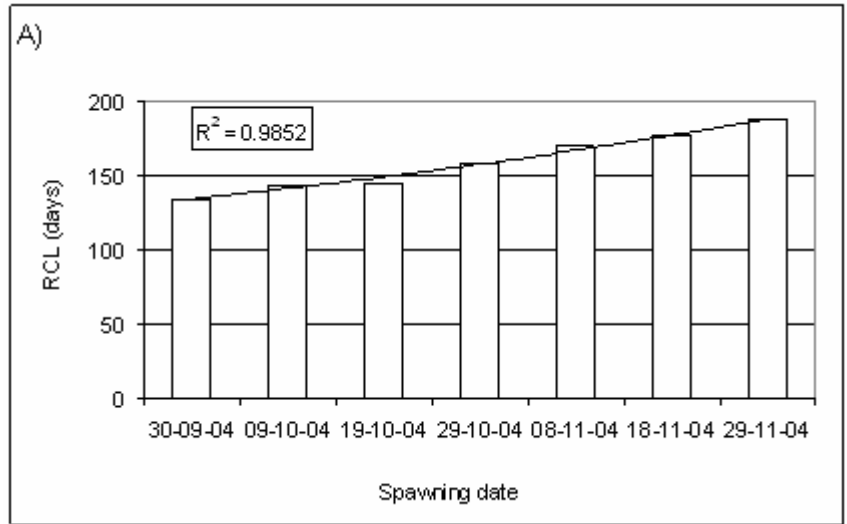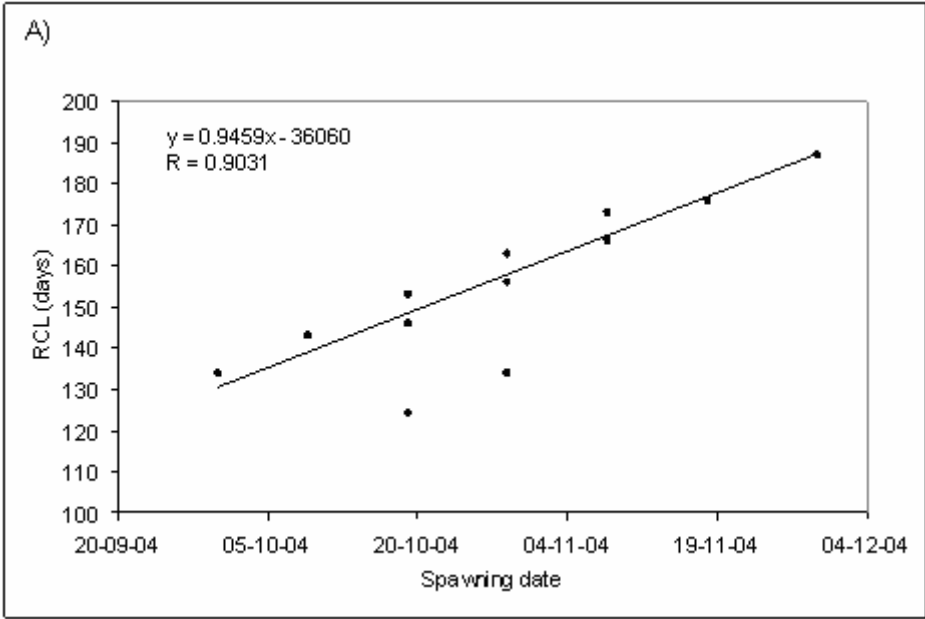
Figure 4. Reproductive cycle length average dynamic in additional or normal cycle of three double cycler population during different spawning season periods a) RCLa Wt-01, additional cycle, spawning season 2004. b) RCLa Wt-02, additional cycle, season 2005. c) RCLn, Wt-03 for 2007 autumn season, estimated from females groups with same spawning date during previous additional cycle during 2006.
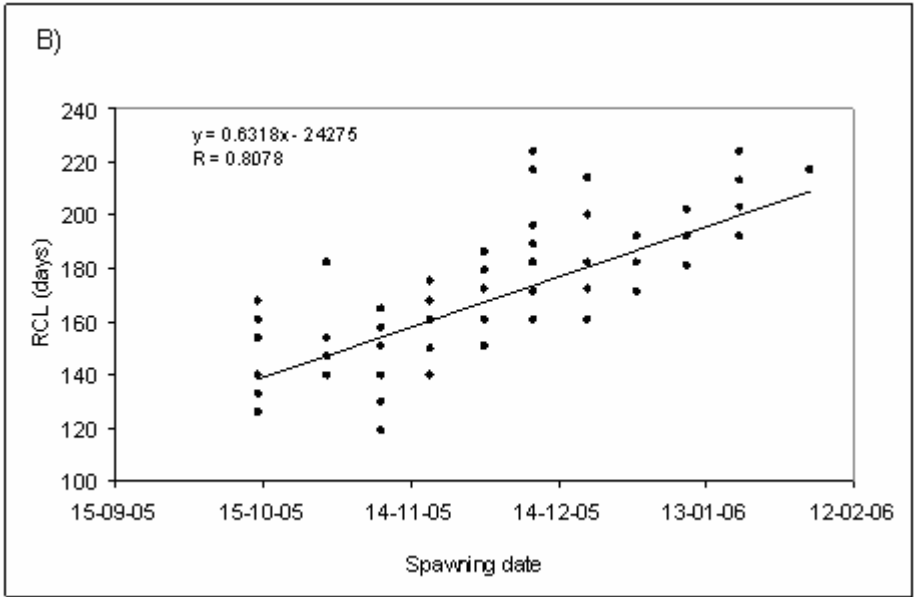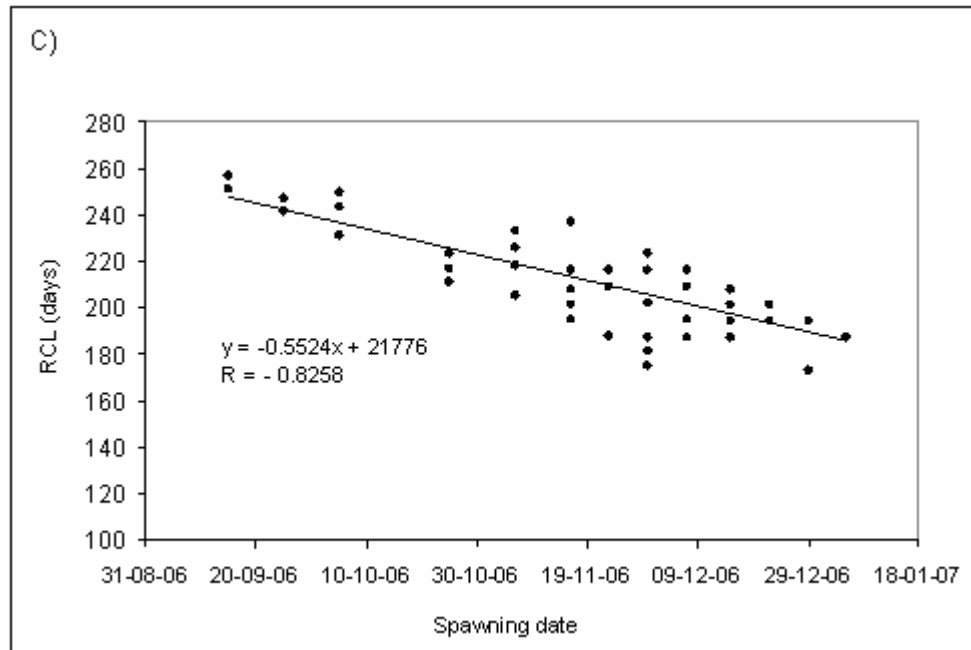
Figure 5. Spawning date correlation vs reproductive cycle length (RCLa or RCLn) in three double cycler population during different spawning season periods a) Wt-01, RCLa, season 2004. b) Wt-02, RCLa season 2005. c) Wt-03 RCLn season 2006-2007 estimated from females groups with same spawning date during previous additional cycle during 2006.
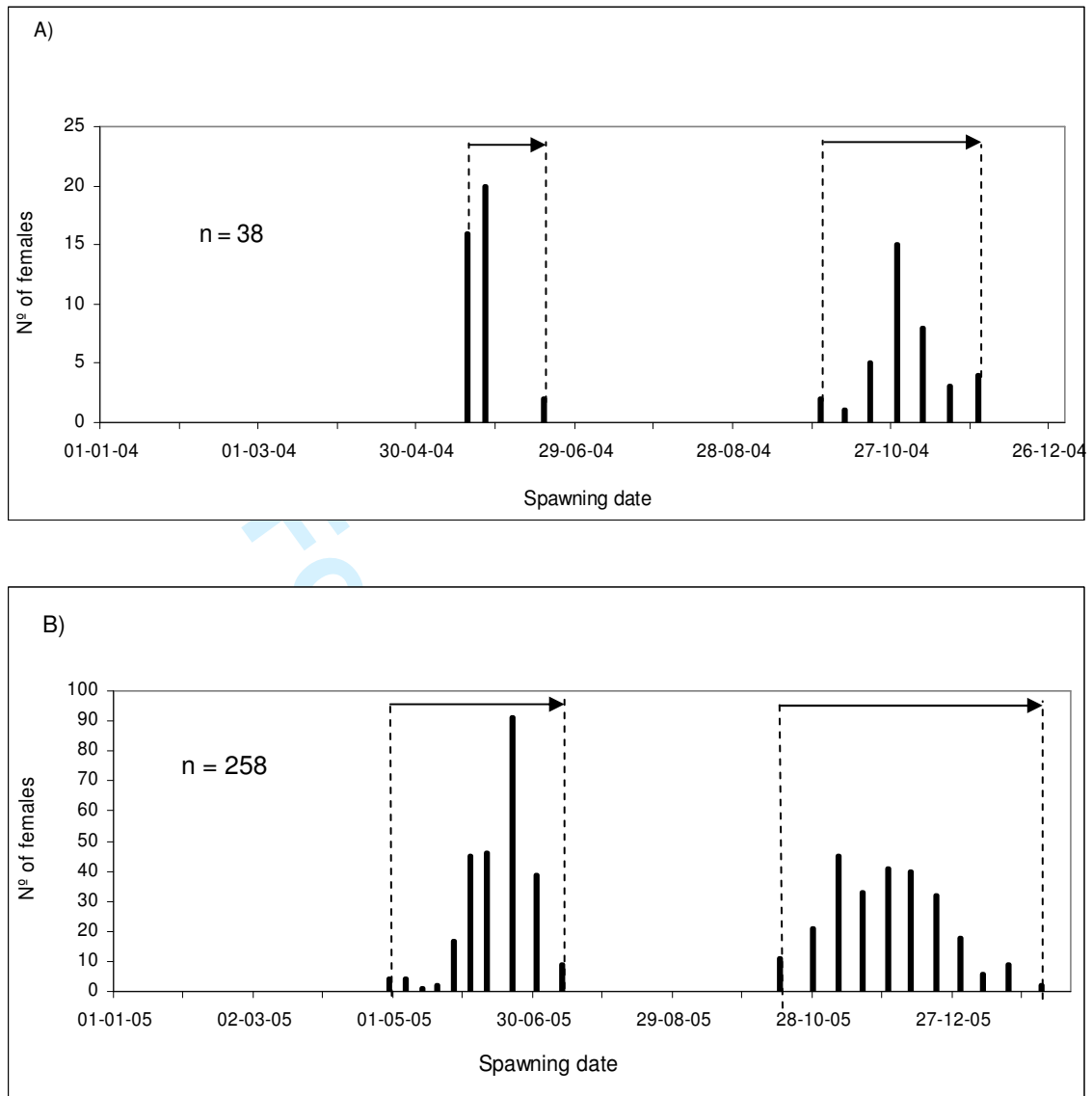
Figure 6. Spawning period length (SPL) of a sample of females of two double cycler population during it normal spawning season and it subsequent additional spawning season : a) Wt-01 during 2004   b) Wt-02 during 2005.