Journal of Applied Ichthyology

J. Appl. Ichthyol. 31 (2015), 529–532

ISSN 0175–8659

Received: April 24, 2014
Accepted: August 27, 2014
doi: 10.1111/jai.12697

Short communication

Dynamics of biannual spawning behavior in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) from southern Chile

By N. Colihueque[1], F. Estay[2] and C. Araneda[3]

[1]*Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Osorno, Chile;* [2]*Piscícola Huililco Ltda., Pucón, Chile;* [3]*Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile*

Introduction

Although the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) is considered a single-time spawner with a restricted annual reproduction cycle (Mylonas and Zohar, 2007), some female broodstocks of this species may display an unusual reproductive periodicity, resulting in a phenomenon characterized by two consecutive annual spawnings as a consequence of biannual spawning behavior (Hume, 1955; Aida et al., 1984; Gall and Crandell, 1992). In these broodstocks (called 'twice-spawners'), the first and second spawning occurs during a normal reproductive cycle (NRC) and an additional reproductive cycle (ARC, respectively). Moreover, the spawning rate in the ARC is usually lower than in the NRC and is highly variable across strains and spawning seasons (Tazaki et al., 1993; Takano et al., 1995; Shrable and Orr, 1998). In addition, the gonadal maturation period previous to the second spawning event, i.e. in the ARC, is a short-term process in these broodstocks, in comparison with the single-time spawner in which gonadal maturation begins a year before ovulation (Elliott et al., 1984; Sumpter et al., 1984). Reproductive performance (Aida et al., 1984; Kincaid, 1985; Tazaki et al., 1993; Takano et al., 1995; Shrable and Orr, 1998), sex hormone profiles (Lou et al., 1984), and gonadosomatic index and gonadal histology (Estay et al., 2012) studies have found that no major reproductive disruptions occur in female twice-spawner rainbow trout. In addition, the examination of the spawning period dynamics indicates that this is longer in the ARC than in the NRC (Tazaki et al., 1993; Takano et al., 1995); furthermore, the inter cycle length (ICL), a parameter that expresses the time required until the occurrence of the additional spawning event for the same female, generally covers a period either from 126 to 180 days (Takano et al., 1995) or from 112 to 217 days (Tazaki et al., 1993).

In spite of this progress, additional studies are needed to compare twice-spawner strains of different origin, to assess whether their particular spawning dynamics follow a general pattern. Therefore, in order to gain an insight into this issue, we examined twice-spawner female broodstocks from a fish hatchery in southern Chile to ascertain the pattern of the NRC, ARC and the ICL following the spawning behavior of individually-tagged breeders. Furthermore, to form a better view of the dynamics of this phenomenon, the relationships between NRC and ARC and between ICL and ARC were also assessed. This study presents the results of the spawning dynamics of biannual spawning behavior in rainbow trout, obtained by studying some characteristic reproductive parameters such as the NRC, ARC and the ICL, derived from the analysis of twice-spawner female broodstock from southern Chile.

Materials and methods

Twice-spawners used for analyses

Two twice-spawner female broodstocks, Wt-01 (n = 38) and Wt-02 (n = 259), were studied, which were obtained from the Piscícola Huililco Ltda rainbow trout fish hatchery located in Pucón, Chile (39°14′29.5″S; 71°50′09.8″W). These broodstocks originated from the cross between females of the Wytheville strain (Wt) and neomales of the Cofradex strain. At the date of the analysis, fish had reached their first maturation event and were at age 3+. All trout were kept in raceway-type ponds supplied with spring water at temperatures of 9 to 11°C, with a water flow of 8–12 L s^{-1} and a culture density of 30 kg m^3. Food for rearing was 5 mm extruded pellets, with a feeding rate of 0.7% live weight day^{-1}. Breeders were individually tagged using electronic passive integrated transponders tags (TROVAN®) to register, unequivocally, the date of their spawn at the NRC and ARC.

Spawning parameters

The spawning parameters analyzed were: (i) date of spawning at first spawning event a year; (ii) date of spawning at the second spawning event a year; and (iii) time needed until occurrence of the additional spawning event. For these parameters, the mean, range, minimum and maximum were calculated in days, considering all females that spawn at NRC and ARC. For each female, the date of the first spawning event in a year was considered as the initial date (day zero) for the timing analysis.

Statistical analysis

The distribution of variables, whether normal or not, was determined according to the Kolmogorov–Smirnov test. Differences between means were calculated by Student's *t*-test, either for dependent samples (within-strain analysis) or independent samples (inter-strain analysis). Pearson product-moment correlation analyses were also carried out to determine the relationship between NRC and ARC and between ICL and ARC. The variables were analyzed for fitting the assumptions of the test (Sokal and Rohlf, 1995) related to normal distribution, homogeneity of variance and absence of correlation between means and variances. All statistical analyses were carried out using the STATISTICA program version 5.1.

Results

The Wt-01 broodstock exhibited a spawning period range of 29 and 60 days during the NRC and ARC, respectively (Table 1). Meanwhile, the Wt-02 broodstock spawning periods during NRC and ARC were 182 and 112 days, respectively, indicating a longer duration of spawning during NRC than in the ARC. In terms of mean duration days, the results corroborate that the spawning period during NRC was shorter than during ARC in Wt-01 broodstock, given that the duration of these periods of 5.2 ± 6.6 days and 32.6 ± 14.7 days, respectively, was significantly different (dependent *t*-test, $P < 0.001$) (Fig. 1). However, in Wt-02 broodstock, similar means for the spawning period during NRC and ARC were observed (47.4 ± 16.9 days and 46.1 ± 24.0 days, respectively; dependent *t*-test, $P > 0.05$), indicating no duration differences. These results show that the spawning period during ARC is longer than in the NRC, or that both might present a similar interval in the biannual spawning strains studied from Chile.

The results of ICL indicate that it was shorter in the Wt-01 than in Wt-02 strain, given the range of 63 and 105 days, respectively (Table 1). However, when the mean values were considered (161.4 ± 15.3 days and 167.3 ± 18.8 days, respectively), no significant differences were observed (independent *t*-test, $P > 0.05$) (Fig. 1). Moreover, the ICL data indicate a similar duration pattern in both strains of 120 to 130 days and 180 to190 days in Wt-01 and from 110 to 120 days and 220 to 230 days in the Wt-02 strain (Fig. 2). These results reveal that a minimum of about 110 to 130 days is needed until the occurrence of an additional spawning event in both strains. Thereafter, the spawns progress over approx. 100 days, to finish at about 220–230 days.

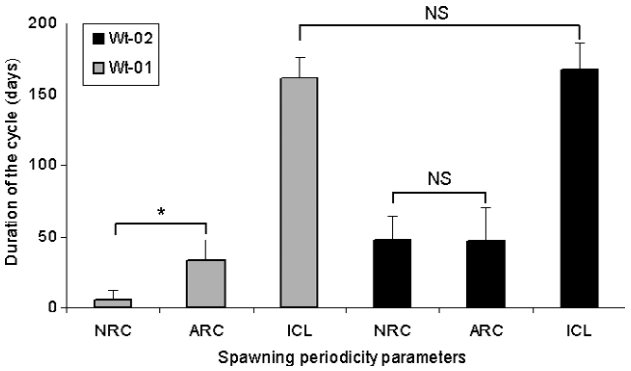

Fig. 1. Duration (days) of normal reproductive cycle (NRC), additional reproductive cycle (ARC), and inter-cycle length (ICL) of two female rainbow trout (*Oncorhynchus mykiss*) broodstocks, Wt-01 (n = 38) and Wt-02 (n = 259), exhibiting biannual spawning behavior. NRC and ARC = duration of spawning season at end of each reproductive cycle; ICL = time elapsed between 1st spawning event at NRC and 2nd spawning event at ARC using the same female. Bar = means ± standard deviation. * = significant differences among means ($P < 0.001$, Student's *t*-test); NS = no significant differences among means ($P > 0.05$, Student's *t*-test)

Correlation analysis reveals a medium-low positive correlation between spawn timing in the NRC and ARC in Wt-01 and Wt-02 strains, but is significant only for the latter ($R^2 = 0.259$; $r = 0.509$; $P = 0.000$); this means that the advance of the spawning timing in the NRC accounts for only 25.9% of the spawning timing variation in the ARC, i.e. NRC spawning date in a twice-spawner partially determines their spawning date in the ARC. However, when the correlation between ICL and spawning timing during ARC was examined, a high, positive significant relationship between both parameters was observed in both strains (Wt-01: $R^2 = 0.816$; $r = 0.903$; $P = 0.000$; Wt-02: $R^2 = 0.625$; $r = 0.790$; $P = 0.000$). These results indicate a high degree of determination, ranging from 62.5 to 81.6%, of the ICL on spawning timing variation during the ARC in

Table 1
Spawning periodicity data of rainbow trout females exhibiting biannual spawning behavior

Twice annually female broodstock spawners	Spawning period	Parameter	Mean ± SD	Range	Min	Max
Wt-01 (n = 38)	19/05/2004 to 17/06/2004	NRC	5.2 ± 6.6	29	0	29
	30/09/2004 to 29/11/2004	ARC	32.6 ± 14.7	60	0	60
		ICL	161.4 ± 15.3	63	124	187
Wt-02 (n = 259)	04/29/2005 to 07/01/2005	NRC	47.4 ± 16.9	182	0	182
	10/14/2005 to 02/03/2006	ARC	46.1 ± 24.0	112	0	112
		ICL	167.3 ± 18.f8	105	119	224

Number (n) of females from broodstocks Wt-01 and Wt-02 studied in trials. Mean and standard deviation (±SD), range, minimum (Min) and maximum (Max) duration in days shown for normal reproductive cycle (NRC) (1st reproductive cycle), additional reproductive cycle (ARC) (2nd reproductive cycle), and inter-cycle length (ICL). NRC and ARC = duration of spawning season at end of each reproductive cycle; ICL = time elapsed between 1st spawning event at NRC and 2nd spawning event at ARC with same female.

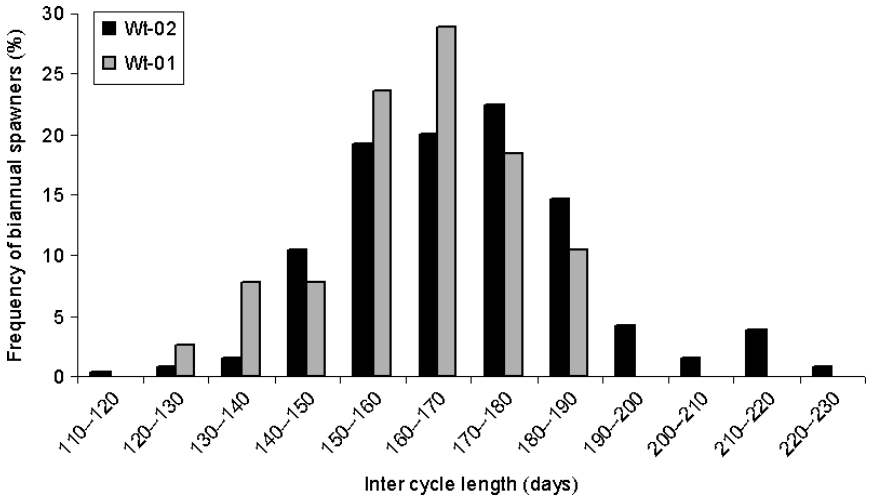

Fig. 2. Inter-cycle length (ICL) distribution of two female rainbow trout broodstocks, Wt-01 (n = 38) and Wt-02 (n = 259), exhibiting biannual spawning behavior. ICL = time required until 2nd spawning event in the additional reproductive cycle (second reproductive cycle) from the 1st spawning event in the normal reproductive cycle (first reproductive cycle) in the same female

twice-spawners. In other words, the data reveal that the ICL is an important factor that determines the spawning date in the additional spawning season.

Discussion

Observations that the spawning period of one of the two biannual spawning strains studied was longer at the normal reproductive cycle than at the additional reproductive cycle agree with Shrable and Orr (1998), but contrast with Tazaki et al. (1993) and Takano et al. (1995), who found that, in several strains of this class from Japan, there was a consistently longer spawning period in the additional reproductive cycle than in the normal reproductive cycle, ranging from 1.5 to 1.8 times. In spite of this, an extensive spawning period at the additional reproductive cycle seems to be coherent with the physiological requirements of the twice-spawner females, in order to ensure the time required to produce mature oocytes in the quantity and quality required for reproduction, although our results suggest that there is no general rule. However, if we compare the duration of inter-cycle length, a parameter that expresses the time required until the additional spawning event occurs, for a particular twice-spawner female, both strains analyzed present a similar pattern. This finding is in accordance with Tazaki et al. (1993) and Takano et al. (1995) and suggests that this parameter could best reflect the dynamics imposed by the oogenesis of twice-spawner females before their second spawning event. Further evidence supporting this phenomenon is the high positive relationship found between the inter-cycle length and the spawning timing in the additional reproductive cycle.

In addition, our data also supports the finding that twice-spawner females require a minimum of 110 to 130 days for the onset of a new spawning event in the additional reproductive cycle, as previously reported (Tazaki et al., 1993; Takano et al., 1995). This spawning timing represents a short interval to develop oogenesis prior to this process, which, in ordinary broodstocks, takes between ten and twelve months (Elliott et al., 1984; Sumpter et al., 1984). Furthermore, it could be expected that this short maturation period may trigger disruption in the oogenesis of twice-spawners, given that the ovarian development of single-time spawners is usually characterized by a synchronic process aimed to yield the development of homogeneous oocytes at the same stage (Mylonas and Zohar, 2007). However, the data support the oogenesis of twice-spawner females, prior to the additional reproductive cycle, indicates a normal maturation pattern (Estay et al., 2012), although some effects during spawning have been observed, such as a higher fecundity (about +900 eggs) that could be related to the smaller eggs (−0.5 mm) produced by these females (Tazaki et al., 1993).

To date, the biannual spawning behavior in the rainbow trout is of unclear origin. However, studies of genetic association with molecular markers linked to spawning time QTLs, suggest that it would be related to this trait (Colihueque et al., 2010). Moreover, environmental factors causing this uncommon reproductive behavior, such as water temperature and chemicals in the feed or water, have also been suggested (Shrable and Orr, 1998); however, there is no evidence to substantiate this. Our work contributes to the clarification of this reproductive phenomenon through the study of their spawning dynamics during the normal reproductive cycle and the additional reproductive cycle. We hope that this information will make a valuable contribution to the application of an appropriate management procedure for cultured twice-spawner broodstocks, aiming to produce eyed-eggs twice yearly with a view to improving the current, or future production of rainbow trout eggs.

Acknowledgements

This study was financed by the Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT) Project no. 1060623. The authors declare that there is no conflict of interest with the commercial entity mentioned in the paper.

References

Aida, K.; Nomura, M.; Lou, S. W.; Hanyu, I.; Tanaka, M.; Tasaki, S.; Ohto, H., 1984: Reproductive activity of a twice annually

spawning strain of rainbow trout. Bull. Jpn. Soc. Sci. Fish. **50**, 1165–1172.

Colihueque, N.; Cárdenas, R.; Ramírez, L.; Estay, F.; Araneda, C., 2010: Analysis of the association between spawning time QTL markers and the biannual spawning behavior in rainbow trout (*Oncorhynchus mykiss*). Genet. Mol. Biol. **33**, 578–582.

Elliott, J. A. K.; Bromage, N. R.; Springate, J. R. C., 1984: Changes in reproductive function of three strains of rainbow trout exposed to constant and seasonally-changing light cycles. Aquaculture **43**, 23–34.

Estay, F.; Colihueque, N.; Araneda, C., 2012: Comparison of oogenesis and sex steroid profiles between twice and once annually spawning of rainbow trout females (*Oncorhynchus mykiss*). Sci. World J., **2012**, 986590.

Gall, G. A.; Crandell, P. A., 1992: The rainbow trout. Aquaculture **100**, 1–10.

Hume, L. C., 1955: Rainbow trout spawn twice a year. Calif. Fish Game **41**, 117.

Kincaid, H., 1985: Development of biannual spawning behavior in a rainbow trout population. In: Salmonid Reproduction: an International Symposium: Review Papers. R. N. Iwamoto and S. Sower (Eds). Bellevue, Washington.

Lou, S. W.; Aida, K.; Hanyu, I.; Sakai, K.; Nomura, M.; Tanaka, M.; Tazaki, S., 1984: Endocrine profiles in the females of a twice-annually spawning strain of rainbow trout. Aquaculture **43**, 13–22.

Mylonas, C. C.; Zohar, Y., 2007: Chapter 15: promoting oocyte maturation, ovulation and spawning in farmed fish. In: The fish oocyte: from basic studies to biotechnological applications. P. J. Babin, J. Cerdá and E. Lubzens (Eds). Springer, Dordrecht, pp. 437–474.

Shrable, J.; Orr, W., 1998: Biannual spawning behavior in three strains of rainbow trout. Available at: http://www.fws.gov/ennis (accessed on 16 April 2014).

Sokal, R. R.; Rohlf, F. J., 1995: Biometry: the principles and practice of statistics in biological research. Freeman and Company, New York, 887 pp.

Sumpter, J. P.; Scott, A. P.; Baynes, S. M.; Witthames, P. R., 1984: Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri* Richardson). Aquaculture **43**, 235–242.

Takano, M.; Nomura, H.; Ootomo, Y.; Tazaki, S.; Tanaka, M., 1995: Reproductive characteristics in the F6 of twice-annually spawning strains of rainbow trout, *Oncorhynchus mykiss*. Bull. Saitama Pref. Fish. Exp. Stat. **53**, 67–73. [In Japanese].

Tazaki, S.; Nomura, H.; Suzuki, K., 1993: Reproduction characteristics of twice-annually spawning strain of rainbow trout, *Oncorhynchus mykiss*. Bull. Saitama Pref. Fish. Exp. Stat. **51**, 63–71. [In Japanese].

Author's address: Nelson Colihueque, Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Avenida Alcalde Fuchslocher 1305, Casilla 933, Osorno, Chile.
E-mail: ncolih@ulagos.cl