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ARTIFICIAL HATCHING SUBSTRATE AND DIFFERENT TIME OF TRANSFER TO STARTFEEDING: EFFECT ON GROWTH AND PROTEASE ACTIVITIES OF THE ATLANTIC SALMON (Salmo salar).

by

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ABSTRACT

Groups of Atlantic salmon (Salmo salar) eggs were hatched in a Californian hatching system with and without an astro-turf artificial substrate, and food was presented at four different points in development. Dry weight development and protease activities were studied. Irrespective of time of transfer the astro-turf reared fry were bigger than the flat screen reared fry at the termination of the experiment. In respect of growth the first and the fourth transfers were clearly suboptimal for the fry from both systems.

INTRODUCTION

The substrate incubator (Bams and Simpson, 1977) was originally constructed to improve the survival rates of artificially reared Oncorhynchus fry used for enhancement purposes. And indeed Bams (1972; 1974) showed that fry from gravel incubators were very similar to naturally produced fry in characteristics such as yolk conversion, size at emergence, growth rate and survival to the adult stage.

Others (Leon, 1975; 79; Ericsson and Westlund, 1983; Hansen and Møller, 1984; Hansen, 1984), have compared incubators with artificial hatching substrates with the traditional flat screened Californian hatching system. These substrates have been demonstrated to improve growth and increase yolk absorption rate during incubation, to prevent development of yolk sac constrictions and to improve growth and survival during feeding. Leon (1979) and Hansen and Møller (1984), however, observed that the flat screen reared fry grew better than the substrate reared in the first period of feeding and suggested that this could be caused by the differences in yolk absorption.

The following investigation was set up to test the effect of different time of transfer to startfeeding of alevins reared with and without astro-turf. As a consequence of the difference in yolk absorption rate, we were especially interested in whether fry reared in a hatching substrate should be trans-

ferred to feeding at a lower age than fry reared on a flat screen.

MATERIALS AND METHODS

Eggs from eight female Atlantic salmon were fertilized and incubated in separate hatching trays at Matre Aquaculture Station.

Experimental conditions

At the eyed stage, the eggs were shocked. Dead eggs were sorted out by an egg sorting machine. The resulting 12 liters of live eggs were pooled to exclude effects due to genetic variation in the material. The eggs were divided into eight groups of 1.5 litre for further incubation. Four of the groups were incubated in standard (EWOS 2003) hatching trays. The other four were incubated in trays in which backless astro-turf was sewn to the perforated aluminium bottom. In this system the eggs were placed on a plastic screen with 3x20 mm perforations which held back dead eggs and therefor prevented them from fouling the substrate (see also Hansen and Møller, 1984).

At intervals from hatching (see table 1.), two groups (one with and one without astro-turf) were both split into two equal sized groups (parallells) and the produced four groups were all transferred to separate feeding tanks (1m). The fry were fed an EWOS ST 40 startfeed nr. 1 in surplus with automatic feeders.

The water input in the hatchery was 10 litres per minute per tray and the temperature varied between 8.8 and 10.4 "C with a mean value of 9.6 "C. The pH varied between 6.0 and 6.8 with a mean of 6.3. During startfeeding the water input was 8 litres per minute per tank and the temperature varied between 11.9 and 13.9 "C with a mean value of 12.5 "C. The water was from 80 to 90% resirculated and was mixed with seawater to a salinity of 6 to 8 ppt to reduce fungus growth in the sedimen-

tation tanks. The seawater gave also a good effect on the pH which was stable at 7.0. However, the seawater also brought the bacteria Vibrio anguilarum which led to increasing mortalities between day 33 and day 38. Consequently the resirculation system was emptied and disinfected with formalin. This lowered the temperature to 8 °C for two days. The Vibriosis was treated with TRIBRISSEN 40% powder (75 mg per kg fish per day) given in the feed. As a further consequence of the Vibriosis, differences in mortalities due to hatchery method or time of transfer could not be registered, and the last transfer which were planned for day 35 were postponed until day 43 at which the fry in the feeding trays had recovered from the Vibriosis.

Sampling and measurements

Both in the hatchery and in the feeding groups, 25 larvae or fry were collected each sampling day for weight measures, the first time at 50% hatching. The fish were collected with a dip-net, anaesthetized with benzocain and preserved in 5% formalin. The larvae were later dissected into yolk and body on preweighed weighing ships. The yolk, body and later whole fry were dried for two days at 60 °C. The dry weight was obtained using an electronic microbalance (d= +/- 0.1 mg). The total weight and the yolk conversion efficiencies, YCE, (Blaxter, 1969) were calculated from the data on body and yolk.

Simultaneously, 30-40 alevins and 10-20 fry were sampled and pooled for the assay of digestive proteases. Because of the very small fish, the whole digestive tract was removed without separating stomach and intestine. The proteases were extracted in 1mM HCl. The extraction method and the assay for proteolytic activities were used according to Rungruangsak and Utne (1981), exept for the homogenization which was made by an Ultra-turrax homogenizer. The peptic and tryptic-like activities were assayed according to Torrissen (1984), at 37.5 °C and 52. 5 °C respectively. The spectrophotometric measurement was done by using a Bausch & Lomb Spectronic 2000. The total

protease activities resulted from the sum of the peptic and tryptic-like activities.

Statistical analyses

The experimental data were statistically analysed by BMDP statistical software (Dixon, 1981). A critical level of 5% were adopted in all tests. The dry weight difference in the body, yolk sac and total weight both in hatchery and during startfeeding were compared using a F-test for equality of variances and later a Student t-test for separate or pooled variances (BMDP3D).

RESULTS

Dry weight development of body and yolk sac

In the hatchery, the difference in body dry weight were statistically significant in favour of the astro-turf reared (ATR) alevins at day 18 ($p=0.000$) and remained significant throughout the hatchery period (Fig. 1).

Both the first and the second transfers led to an increase in body growth rate of the flat screen reared (FSR) alevins relative to the FSR hatchery groups (Fig. 1A,B). The same transfers led to a decrease in body growth rate of the ATR alevins. The third transfer was, however, followed by a decrease in body growth rate relative to the hatchery groups for alevins in both systems (Fig. 1C).

The ATR alevins had a higher yolk absorption rate in the period from day 18 until day 28 giving a significant difference in yolk weight between the systems on the latter day (Fig. 2). However, from day 28 and throughout the hatchery period the FSR alevins had a slightly higher absorption rate than the ATR alevins. The difference in yolk weight was consequently not significant on the days 38 and 43.

The yolk absorption rate was higher in the startfeeding tanks than in the hatching systems irrespective of time of

transfer. Moreover, the earlier the alevins were transferred the greater was the subsequent deviation from the hatchery curves.

As shown in table 3, the ATR alevins had higher YCE than FSR alevins irrespective of time of transfer. Moreover, the first transfer is clearly suboptimal both for the ATR and FSR alevins in respect of the YCE. For the ATR alevins the third transfer proved better than the second and the fourth. For the FSR alevins no differences in YCE was found between the last three transfers.

Growth during feeding

The dry weight development of alevins and fry during the experimental period are presented in Figure 3.

Irrespective of time of transfer and exept for in the relation between the smallest ATR group (astro-turf reared first transfer,ATR1) and the biggest FSR group (FSR3) the differences between the hatching systems was significant in favour of the ATR groups at the termination of the experiment on day 58 (Table 2.). On this day the groups ranged in the order ATR2, ATR3, ATR4, ATR1, FSR3, FSR1, FSR4 and FSR2 with ATR2 as the biggest group. As shown in the results for the ATR groups, the second transfer proved better than the third transfer, a result which was reversed in the FSR groups.

Protease activities

The digestive proteases of the alevins in the hatchery were well developed after 38 days (Fig. 4). The enzymatic activities increased after the first and the second transfers to startfeeding, but not after the third one. For the first transfer, the total activities increased 0.80-3.66 folds in the ATR groups and 1.02-12.45 folds in the FSR groups upto day 38 and for the second transfer, they were 1.34-2.00 and 1.61-6.10 folds respectively. The enzyme activities of the fry of the first, second and third transfer were similar to those of alevins in the hatchery on day 43.

At the end of the experiment (day 58), a difference in total protease activities between the FSR and ATR fry was observed in the groups from the third and the fourth transfers but not in the first and the second transfers (Fig. 4B).

Irrespective of the time of transfer, the enzyme activities on day 58 were similar in all ATR groups. As shown in Fig. 4A, the total specific activities seemed to be stable from day 28 after hatching. After the first and the second transfer, the specific activities increased 1. 22 and 1.65 folds, respectively, in FSR groups, and 2.05 and 0. 69 folds in ATR groups, on day 28. From day 38 until day 43, the specific activities decreased and had similar levels as in the hatchery. On day 58, there were no alevins left in the hatchery to be compared, but the total specific activities were also similar in all the transferred groups. As expected there was no difference in the total specific activities between the FSR and ATR groups.

DISCUSSION

Dry weight development of body and yolk sac

The difference in growth rate of the FSR and ATR alevins during hatchery incubation is in accordance with the results of Marr (1965), Leon (1975) and Hansen and Møller (1984). The non-supportive flat screen fail to satisfy the alvins preferred stability in the vertical level (Marr, 1963; Bams, 1969). This is compensated by high swimming activity and the FSR alevins consequently convert less yolk to body tissue.

Lowered yolk aborption rate of FSR alevins relative to substrate incubated alevins was first observed by Leon (1975), and is later confirmed by Hansen (1984), Hansen and Møller (1984) and Hansen and Torrissen (1984). Hansen and Møller (1984) attributed the lowered yolk absorption rate to the high activity stress of FSR alevins. In this experiment the difference was, however lower than expected.

The difference in the yolk absorption rate between the

hatchery groups and the feeding groups is a function of the difference in temperature between the two as an increase in temperature is known to increase the yolk absorption rate (Hamor and Garside, 1977; Peterson, 1977; Heming, 1982). The difference in temperature will also tend to increase the difference in yolk weight between the groups in the hatchery and the feeding tanks as a function of increasing time of exposure to the different temperatures as seen in the results from the different time of transfer.

The temperature caused difference in yolk absorbtion rate also increased the body growth rate of the first and second transferred FSR alevins relative to the hatchery groups. These transfers involved no substrate changes for the FSR alevins as both their hatching trays and their feeding tanks are flat bottomed. For the ATR alevins, however, the transfers involved a change from a supportive to a non-supportive substrate. This highly increased the activity of the alevins and the higher absorbtion rate could not fully compensate for the higher maintenance level. As the alevin size increased, the activity and consequently the maintenance level also increased. The third transfer therefore as all transfers of the ATR alevins does not increase the growth rate of the FSR alevins.

Growth during feeding

In the first period after transfer to startfeeding and prior to the initiation of feeding, the yolk is the only energy source of the fry. The initiation of the first feeding is probably related to a reduction in available yolk nutrients (Dill, 1970 (cited in Twongo, 1975); Twongo, 1975). After the first feeding has been initiated more and more of the nutrients will come from the feed. This overlap in yolk absorption and feeding is presumably of survival value (Wallace and Aasjord, 1984) and is reported for brook trout (Leach, 1924), chum salmon (Disler, 1953), rainbow trout (Twongo, 1975), chinook salmon (Heming et al., 1982) and arctic charr (Wallace and Aasjord, 1984).

Hurley and Brannon (1969) found that exogenous feeding had no enhanching effect on the growth of sockeye salmon (Oncorhynchus nerka) alevins. Palmer et al., (1951) found, however, that feeding during yolk absorption of the sockeye salmon and chinook salmon (O. tschawytscha) had this effect. The latter observation is in accordance with other results on chinook salmon (Heming et al., 1982) and Arctic charr (Wallace and Aasjord, 1984). Such an effect could explain the higher YCE´s of the alevins of the third transfer. Normally one would expect that alevins transferred to a higher temperature would experience a lowering of the YCE (Heming, 1982; Wallace and Aasjord, 1984) due to a higher maintenance level. Body growth rate due to exogenous feeding will, however, increase the YCE and obscure the effects of the higher temperatures.

The weight difference in favour of the ATR fry on day 58 is partly a result of difference in weight at transfer, partly a result of a higher growth rate of ATR during startfeeding. The latter effect is in accordance with the results of Leon (1975; 79), Hansen and Møller (1984) and Hansen (1984) and is probably a result of a bigger and stronger fry, a more advanced yolk absorption and a more advanced morphological development (Hansen, 1984).

It is uncertain to what degree the growth of the different groups was affected by the Vibrio attack. However, on day 43 the ATR3 group was in good growth and was significantly heavier than all the other groups irrespective of hatching system and time of transfer. It seems that the drop in the growth rate of the ATR3 group in the last period of feeding was caused by the pathogens, an inferrance which also was supported by the observations on protease activities. This also indicate that the at least the ATR3 group could prove better than would appear from the data on day 58. Moreover, in respect of growth it seems that the first and the fourth transfers are clearly suboptimal for the fry from both systems.

Protease activities

The total specific activities was stable from day 28 after hatching (Fig. 4A) but the enzyme synthesis increased gradually during the experimental period (Fig. 4B). Even if the feeding increased either the specific activities or the total amount or both, this did not lead to a higher activity later in the feeding period. This is seen in the fact that the activities in the late stages of feeding were not different from the corresponding activities in the hatchery.

The decrease in the total specific activities in the startfeeding groups from day 38 throughout the experiment might be because of the Vibriosis. The total specific activities of the first and second transferred groups after day 28 should be either increased or stabilized because the levels of the specific activities at 2.71-7.45 µmol tyrosine per hour and mg protein are normal for immature Atlantic salmon (Torrissen and Torrissen, 1984).

Differences in total protease activities was found between the FSR and ATR fry from the third and the fourth transfers but not from the first and the second ones. This effect is expected as the difference induced by the substrate will develop only after some time of incubation.

CONCLUSION

From this experiment it cannot be concluded that ATR fry should be transferred to startfeeding at a lower age than FSR fry. However, further experiments are nessessary to investigate the effects of transfer in the period from day 22 to day 43 (or corresponding period with other incubation temperatures).

However, the astro-turf incubation favour growth both of alevins during the hatchery incubation and of the fry during feeding. It can also concluded that the early and late transfers are suboptimal in respect of growth for fry from both systems.

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Table 1: Experimental setup.

DAY 0	DAY 19	DAY 22	DAY 28	DAY 43
FS 1	TANK 1			
	TANK 2			
AT 1	TANK 3			
	TANK 4			
FS 2		TANK 5		
		TANK 6		
AT 2		TANK 7		
		TANK 8		
FS 3			TANK 9	
			TANK 10	
AT 3			TANK 11	
			TANK 12	
FS 4				TANK 13
				TANK 14
AT 4				TANK 15
				TANK 16

Table 2: Results from the statistical testing of the data on day 58. All numbers are probability values.

	FS1	FS2	FS3	FS4	AT1	AT2	AT3	AT4
FS1	-	0.21	0.28	0.42	0.07	0.00	0.00	0.00
FS2	-	-	0.01	0.54	0.00	0.00	0.00	0.00
FS3	-	-	-	0.02	0.36	0.00	0.00	0.03
FS4	-	-	-	-	0.00	0.00	0.00	0.00
AT1	-	-	-	-	-	0.00	0.01	0.21
AT2	-	-	-	-	-	-	0.53	0.03
AT3	-	-	-	-	-	-	-	0.13

Table 3: Calculated yolk conversion efficiencies for the period from day 0 till day 43 for the different systems and time of transfer.

Time of transfer	ATR	FSR
Day 43	64.5	57.0
Day 28	72.8	58.2
Day 22	65.2	57.5
Day 19	59.1	51.5

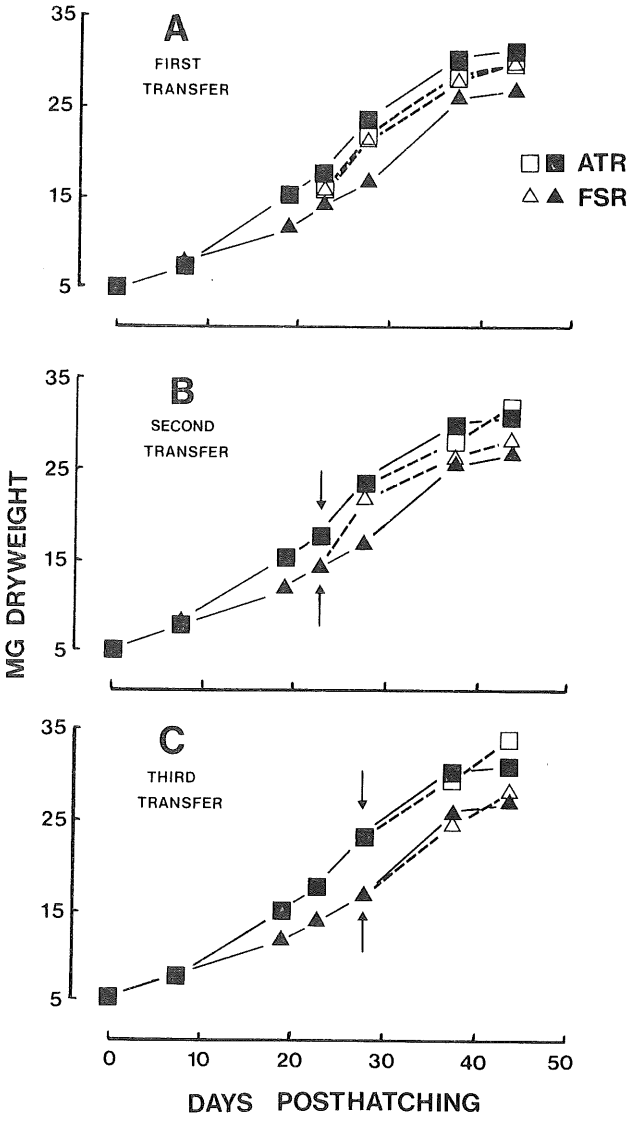

Fig. 1. Dryweight development of body (totalweight - yolkweight) of alevins during the experiment. The time of transfer is indicated with arrows. Hatchery period, solid symbols; feeding period, open symbols.

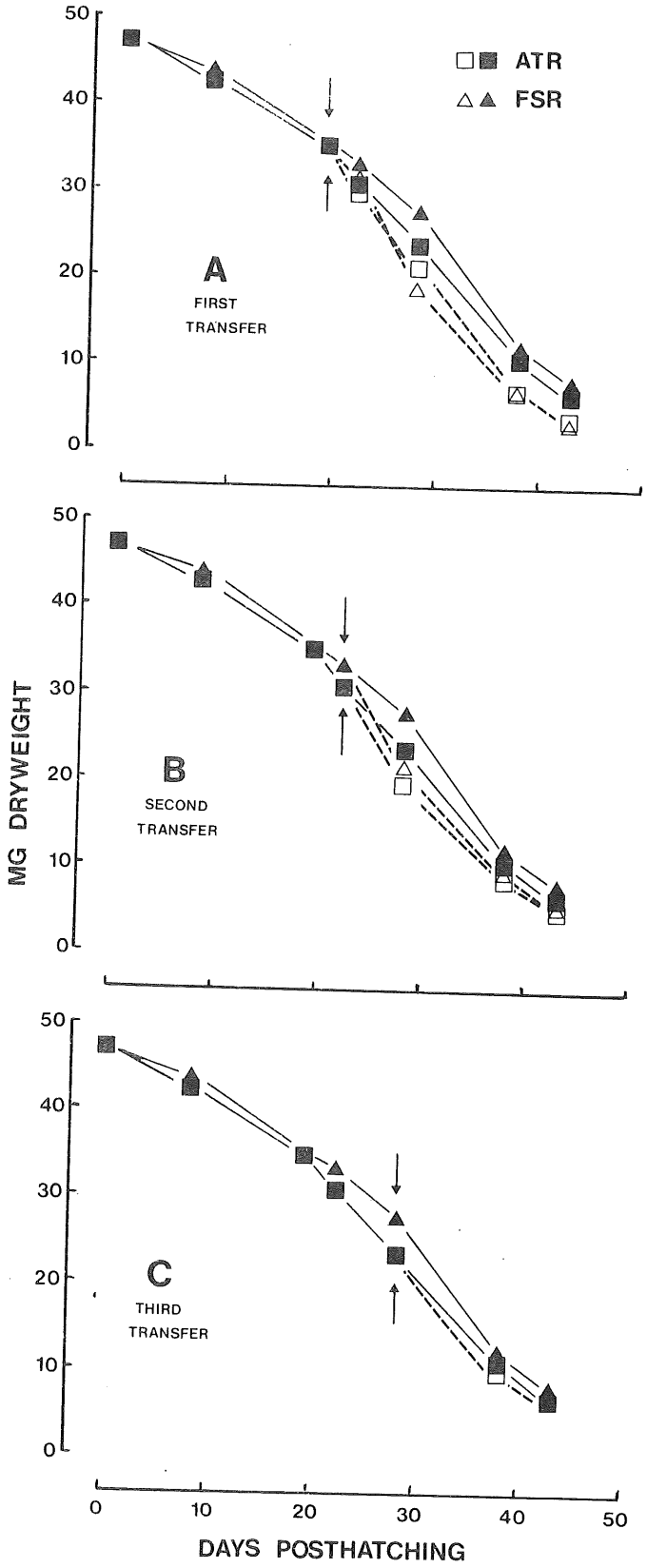

Fig. 2. Dryweight development of yolk during the experiment. The time of transfer is indicated with arrows. Hatchery period, solid symbols; feeding period, open symbols.

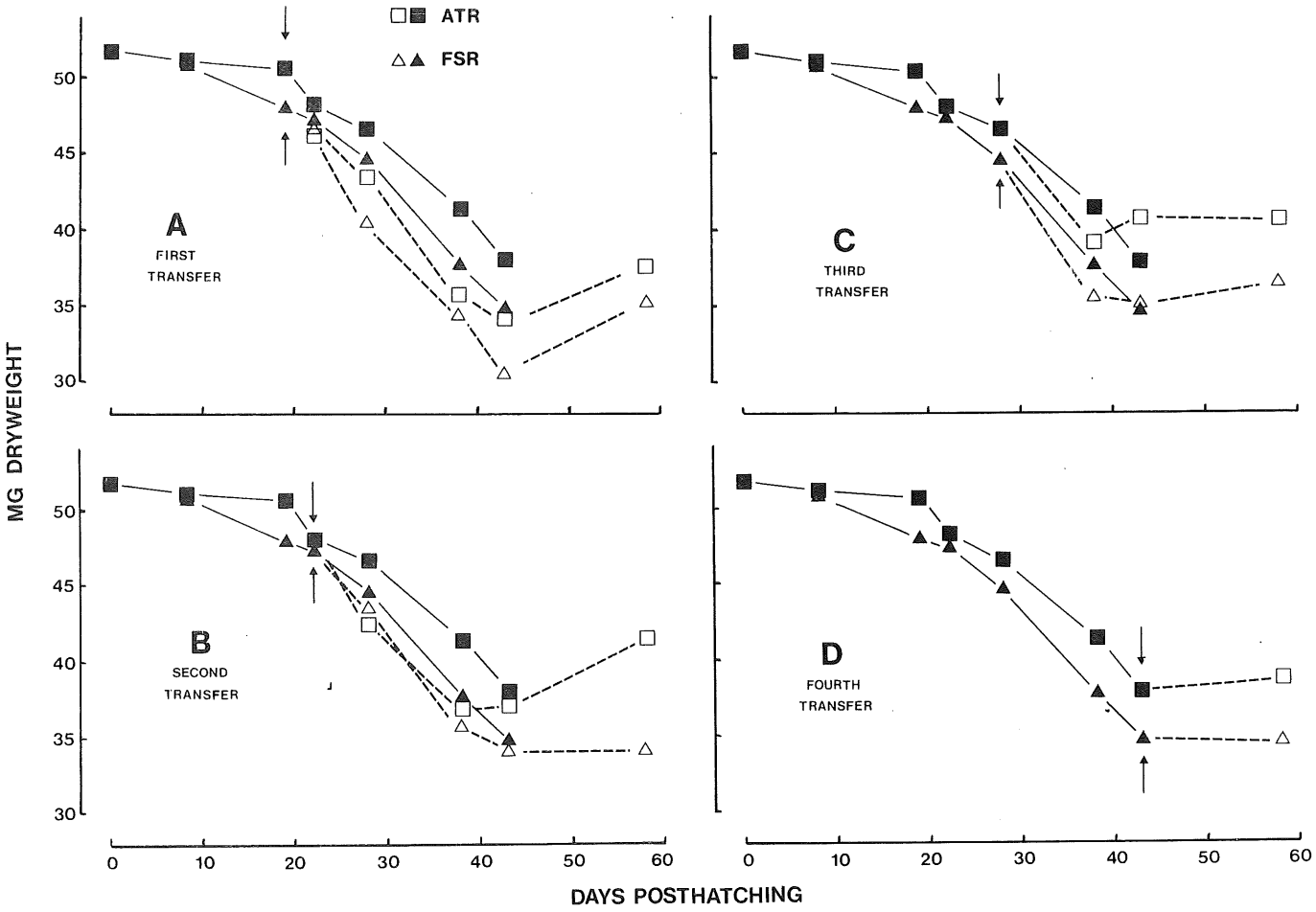

Fig. 3. Dryweight development of alevins or fry (totalweight) during the experiment. Hatchery period, solid symbols; feeding period, open symbols.

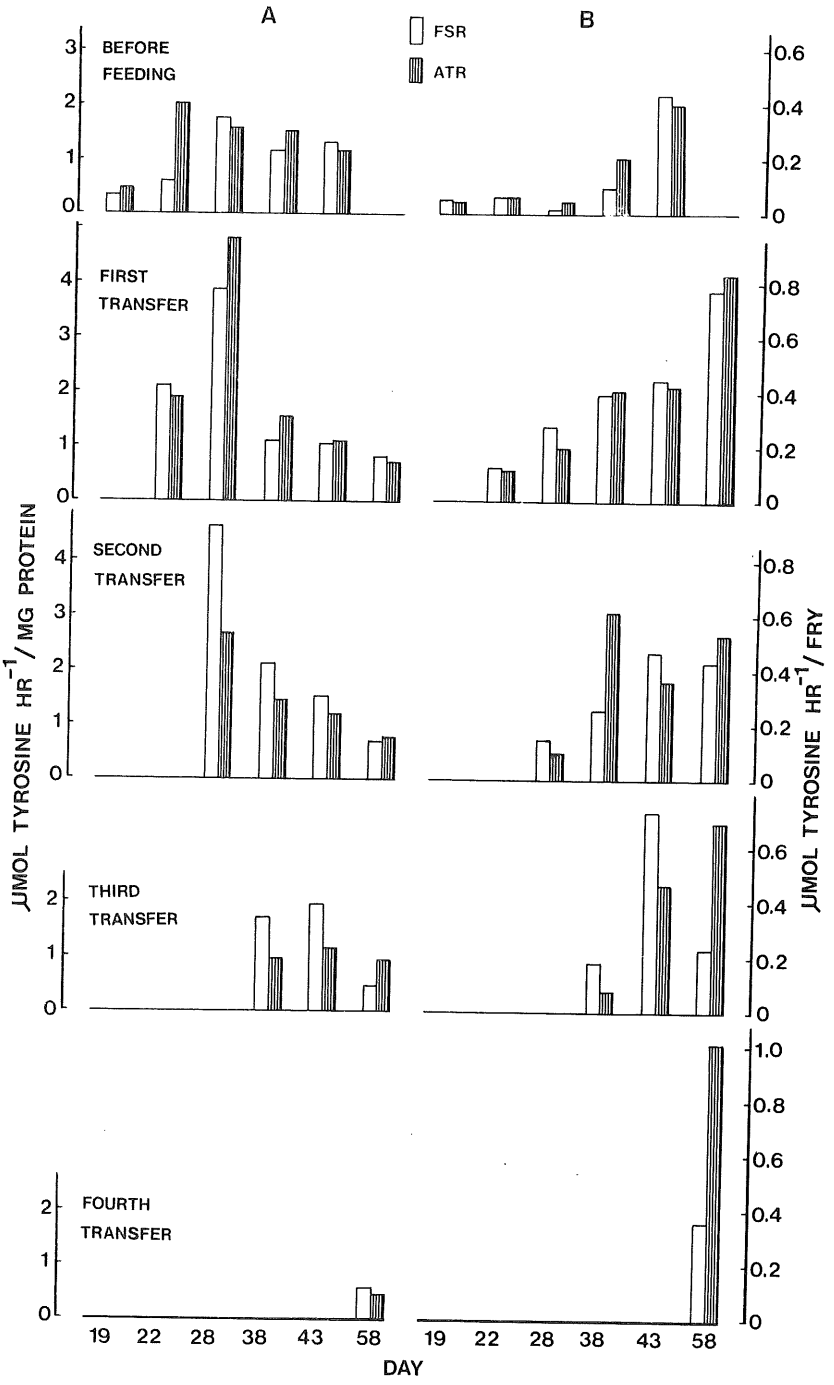

Fig. 4. The development of protease activities in the digestive tissues of Atlantic salmon fry before and after different time of transfer to startfeeding.