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PRELIMINARY APPRAISAL OF AN EXPERIMENT INVESTIGATING THE EFFECTS OF PANCREAS DISEASE, INFECTIOUS PANCREATIC NECROSIS AND DOUBLE INFECTION ON INDIVIDUAL GROWTH RATES IN ATLANTIC SALMON

by

R S Raynard and D A Smail

SOAFD Marine Laboratory
PO Box 101, Victoria Road
Aberdeen, AB9 8DB
Scotland, UK

Bundesforschungsanstalt
Bibliothek
für Fischerei, Hamburg

SUMMARY

The effect of pancreas disease (PD) and infectious pancreatic necrosis (IPN) on the growth of Atlantic salmon in sea water was examined with the agents alone and together. The experiment used individually marked fish of approximately 300 g mean weight at the start (January, temperature = 6.5°C) and 500 g at the end of the experiment (May, temperature = 9°C). The PD infected group developed typical signs of PD with up to 50% of fish showing complete acinar cell necrosis in association with severe growth depression. Growth of the PD affected group returned to normal after 50-60 days but mean weights were lower than controls and were equivalent to 20 days loss of growth. IPN produced only mild pathological signs in the pancreas and moderate virus titres in the pancreas/caeca and kidney, without any effect on growth rate. There was no synergistic effect of IPN and PD. On the contrary, prior infection with IPN reduced susceptibility to subsequent infection with PD. PD infection of IPN carrier fish caused some clearance of IPN virus as measured by lower pancreas/caeca titres.

INTRODUCTION

Infectious pancreatic necrosis (IPN) is a viral disease of salmonids which can cause mortalities in Atlantic salmon (*Salmo salar*) yolk-sac and first-feeding fry (Hill, 1982; Smail *et al.*, 1986). The effect of IPN virus on the development of Atlantic salmon smolts and post-smolts is less certain. In Norway, several reports associate mortalities of Atlantic salmon post-smolts with IPN in sea water (Christie *et al.*, 1988; Krogsud *et al.*, 1989) and in Scotland an increase in the incidence of mortality and poor growing of post-smolts in association with IPN was noted (Smail *et al.*, 1992).

The significance of the association between IPN and the poor performance of salmon reported in the above mentioned studies is difficult to determine since animals had been recently transferred to sea water. At transfer time salmon are undergoing physiological

adaptation to sea water and may be vulnerable to environmental stress resulting in increased susceptibility to endemic infectious diseases. Despite the associative findings, we know of no published experimental studies demonstrating a causal connection between IPN and the performance of Atlantic salmon in sea water.

Pancreas disease (PD) affects Atlantic salmon in sea water and is characterised by the degeneration and widespread loss of acinar cells of the exocrine pancreas (Munro *et al.*, 1984; McVicar, 1987). Fish affected by PD may stop feeding and emaciation of fish affected by PD is commonly reported. Although of unknown cause, PD can be experimentally transmitted and is likely to be caused by an infective agent, probably a virus (Raynard and Houghton, 1993). Experimental studies have shown that PD causes reduced growth rates, without mortality, of Atlantic salmon in sea water (Raynard, unpublished data).

Pancreas disease has been proposed as a disease which may interact with IPN to the detriment of salmon health. The possibility of a synergistic effect between PD and IPN affecting the health of salmon post-smolts has been considered by Poppe *et al.* (1989). Smail *et al.* (1992) also suggested PD as a possible factor affecting post-smolts showing poor condition and mortality in association with IPN.

The aim of our study was to experimentally investigate the effects of the two diseases, IPN and PD, on the growth rate of individually marked Atlantic salmon in sea water and to determine the effect of PD on the growth rate and IPN virus titre of tissues in fish which had previously been infected with IPN. Additionally, a histological assessment was made of the effects of the diseases both separately and together.

MATERIALS AND METHODS

Fish

Atlantic salmon reared at the fish cultivation unit of the Scottish Office Agriculture and Fisheries Department (SOAFD), Aultbea, Ross-shire, Scotland, were transported to the Marine Laboratory aquarium, Aberdeen. Fish were maintained in 1 m diameter tanks containing 350 l of sea water, supplied at *ca* 10 l min^{-1} $tank^{-1}$. Fish were fed (Mainstream diets, BP Nutrition) to satiation during the hours of artificially maintained daylight. The water temperature at the time of the first injection was 6.5°C (February) and rose to 9°C by the end of the experiment (May). Prior to all experimental procedures fish were anaesthetised using ethyl-4-aminobenzoate (benzocaine).

Growth Rates

Growth rates were calculated as the daily percentage increase in weight by the formula, % growth rate = (weight at time b - weight at time a) ÷ weight at time a x 100 ÷ days between time a and b. Growth rate was expressed as % body $weight.day^{-1}$.

Statistical Analysis

Minitab was used for all the analyses. One-way analysis of variance was used to compare population means of fish weights and growth rates between treatments. T tests were used to compare virus titres. Correlation coefficients were used to test for the association of

virus titre with fish weights and growth rates. Where probability (p) values were less than 0.05 the null hypothesis was rejected.

Pancreas Disease, Transmission and Diagnosis

Pancreas disease was experimentally transmitted to salmon by the method of Raynard and Houghton (1993) using intraperitoneal injections of 0.2 ml of kidney homogenates at a dose of 3 µg protein.g^{-1} body weight. Diagnosis of pancreas disease was made by histological examination of the pancreas (Raynard and Houghton, 1993). Fish were classified as affected when the normal acinar arrangement of the exocrine pancreas secretory cells had become transformed into one of total apparent necrosis and when no zymogen containing cells (eosinophilia) could be observed.

Growth of IPN Virus

The strain of IPN used in these experiments was IPN (serotype Sp, strain Sh) which had been isolated from salmon post-smolts having high IPN virus titres in association with poor growth and mortality. A recent strain of IPN Sh (Ross, 1991; Pryde *et al.*, 1993) was grown from frozen stock on CHSE cells at low multiplicity of infection. Five roux flasks of CHSE cells were harvested at day 5 when viral CPE was complete and the cells sedimented. Virus was precipitated from the supernatant using polyethylene glycol (PEG) by the method of Dixon and Hill (1983). The PEG-virus precipitate was sedimented and resuspended in 40 ml HBSS, which was titrated on CHSE cells in 24 well-plates. The virus isolate had been passaged through cells three times by the time it was injected into fish.

IPN Virus Titration

a) Kidney

Approximately 0.5 g of head kidney was dissected aseptically, weighed and homogenised in 19 volumes of Hanks balanced salt solution (HBSS 1x) using a stomacher 80 (Seward Medical). The homogenate was sonicated in a water bath sonicator (Heat systems, Farmingdale, NY, model ZL 2020) at 550 W for one minute. The sonicated homogenate was clarified by sedimentation at 3,000 g for 15 min and the supernatant passed through a Millipore (HV) 0.45 µm low protein binding filter. An aliquot of filtrate was titrated on 90% confluent CHSE cells in 24-well plates using final dilutions of the inoculum from 2×10^{-1} to 2×10^{-6} and 0.5% agarose/Mem-2 overlay. Cultures were fixed and stained at 55 hours post-infection and plaques enumerated.

b) Pancreas/caeca

Approximately 1 g blocks of pancreas/caeca were dissected, weighed and homogenised in 49 volumes of HBSS by stomacher as above. Virus titre was assessed as plaque forming units (pfu) by the method described above.

Infection of Fish with IPN

Fish were injected with IPN/Sh in HBSS at 5×10^5 pfu.g^{-1} fish. The dose was volume adjusted for varied fish weights and controls received injections of HBSS.

Experimental Details, Sequence and Timing of Experimental Infections

Following transfer to the aquarium, fish (mean weight 265 g) were divided between eight identical tanks, individually dye-marked by the method of Johnstone (1983) and acclimated for a period of two months. Fish were monitored during the acclimation period to ensure uniformity of mean weight and growth between tanks.

The numbers of fish contained in each tank and treatments carried out are shown in Table 1.

RESULTS

Fish Growth

Figures 1 and 2 show the mean growth rates and mean weights of fish versus time for tanks 1, 2, 3, 5, 6, and 7. The data for tank 8 has not been included since this tank initially contained a greater number of fish than the other tanks. However, the growth and mean weight of fish in tank 8 was not different to the other tanks containing IPN infected fish (6 and 7).

There was no significant difference ($p>0.05$) in either mean weights or growth rates between tanks up to the time of the first injection. A comparison of growth rates 15 days after the first injection revealed significant differences ($p=0.03$) with the PD infected fish showing a slight reduction in growth rate. Growth rates reduced further in the PD affected fish such that 28 days after the first injection the mean growth rate was 0.21% body weight.day^{-1} compared with 0.57 to 0.68% body weight.day^{-1} for the other fish populations. This depression of growth, caused by PD, resulted in four fish developing negative growth rates. The change to negative growth rates was not seen in any fish in the other tanks. The mean growth rate of the PD affected population of fish in tank 1 recovered slowly but remained depressed at 42 and 55 days post-infection. This PD affected population took 61 days from the time of minimum growth 0.21%.day^{-1}, to achieve growth rates comparable with the control (tank 2).

The group of fish infected with IPN (tank 5) did not develop different mean growth rates. This lack of effect of IPN on growth was supported by the two groups of fish (tanks 6 and 7) which up to the time of the second injection were only infected with IPN and showed similar growth rates to the control tanks (2 and 3, tank 3 of use as a control replicate upto the time of the second injection).

Thirteen days after the second injection, the growth rates of fish which were first control injected and then infected with PD (tank 3) were not different to the control, IPN-only and IPN with PD populations (tanks 2, 5, 6, 7 and 8). By the time of the next observation, 27 days after the second injection, the mean growth rate of fish in tank 3 (first injection control, second injection PD) had declined from 0.59% body weight.day^{-1} to -0.05% body weight.day^{-1}. At the same time, the growth rates of fish first injected with IPN and secondly injected with PD (tanks 6 and 7) declined to means of 0.38 and 0.34% body weight.day^{-1} respectively. The mean growth rates 27 days after the second injection were significantly different ($p<0.001$) with a greater reduction in growth rate for fish which had received control and then PD injections (tank 3) compared to fish which had received injections of IPN followed by PD (tanks 6 and 7). The proportion of fish showing negative

growth rates was 10/18 for tank 3, 5/29 for tank 6 and 3/18 for tank 7. Therefore, the effect of PD on growth was less when fish had been previously infected with IPN. By 45 days after the second injection the mean growth rates between tanks were different ($P<0.001$). The growth rates of fish in tanks 3, 6 and 7 had started to recover but were still lower than the control group (tank 2) and the group only infected with IPN (tank 5).

Changes in the mean weights of fish with time are shown in Figure 2. The fish infected with PD at the first injection are the only group of fish which showed consistently lower weights than the control fish. Reduced weight for these fish was noted 13 days after the PD injection and coincided with the decline in growth rate. The mean weights were not significantly different until the time of the last measurement although the probability values of 0.067 and 0.06 at, respectively, 55 and 69 days following the first injection approach significance. The fish infected by PD at the first injection continued to have reduced weights even after the recovery of growth rate indicating that PD had a long-term effect on the weight of fish. By the time of the last observation the groups of fish which had been infected with PD at the second injection (tanks 3, 6 and 7) had mean weights lower than the control (tank 2) and IPN-only infected fish.

IPN Virus Titres

No IPN virus was isolated from fish in the control tanks. Figures 3 and 4 show the titre of IPN virus in kidney and pancreas/caeca versus time. Titres of approximately 10^5 pfu.g^{-1} tissue were achieved through the injection. For fish infected only with IPN the titres for kidney and pancreas/caeca were similar with a decline in titre of approximately 1.0-1.5 $\log_{10}$ from day 28 to day 69 post-infection.

Infecting IPN positive fish with PD had no effect on the titre of IPN virus in the kidney (Fig. 3). However, for pancreas/caeca, the titre of IPN virus was lower 42 days following infection with PD (Fig. 4).

Correlations were investigated between IPN virus titre in kidney and pancreas/caeca and the growth rate and body weight of individual fish whenever titres of IPN virus were measured. No significant correlations ($P>0.05$) were observed for the groups of fish which had only been infected with IPN (tanks 5 and 8). The only significant correlation observed was for the titre of IPN virus in the pancreas/caeca against growth rate for fish first infected with IPN followed by PD (tank 6 $P<0.01$ see Fig. 5 and tank 7 $P<0.05$). IPN virus titre in the pancreas/caeca was positively correlated with growth rate.

Histopathology

Table 2 summarises the histological results. Livers and kidney were not affected by IPN (tanks 7 and 8). Fifteen days after infection with IPN (tank 8) a few exocrine pancreas acinar cells were vacuolated and appeared shrunken, few necrotic areas were noted. No samples were available for 28 and 42 days after the first injection. At 55 and 69 days after infection with IPN, pancreas pathology was restricted to the shrunken appearance, indicating necrosis, of a few acinar cells. The exocrine pancreas of five out of 29 fish which were sampled 89 days after infection with IPN (tank 5) had a few small areas of necrotic acinar cells.

Fish which had previously been control injected and were infected with PD at the second injection developed some large areas of acinar cell loss in the exocrine pancreas 13 days

after the injection. The number of fish affected increased up to the termination of the experiment with increasing severity of acinar cell loss and 50% of fish being diagnosed as having PD. Diagnosis of PD was made, according to Raynard and Houghton (1993), when total loss of exocrine pancreas acinar cells was observed throughout the whole of the pancreas present in a section. At the termination of the experiment, 89 days after the first injection, only one of the fish infected with PD at the first injection (tank 1) had pancreas disease with total absence of acinar cells. Most of the remaining fish showed a pancreas of normal appearance with the rest showing intermediate levels of acinar cell loss. Since this group of fish had suffered decreased growth rates which had returned to normal at the time of histological sampling, it is assumed that pancreas recovery had taken place following a high incidence of pancreas disease.

For the IPN-positive fish subsequently infected with PD, the nature of the exocrine pancreas pathology was broadly similar to that seen in fish first injected as controls but which received PD at the second injection. However, the proportion of fish which were diagnosed as having PD was lower in the groups of fish infected first with IPN and secondly with PD (20% tank 6; 10% tank 7) compared to the group of fish only infected with PD (50% tank 3). This reduction in the incidence of histologically diagnosed PD in fish infected with IPN is consistent with the observation of higher growth rates in fish infected first with IPN and secondly with PD compared to fish which were previously control injected before infection with PD.

No histopathology of the exocrine pancreas was noted in fish which only received control injections. All liver and kidney tissue appeared normal.

DISCUSSION

Studies of fish growth usually include replicate tanks in order to assess tank-dependent effects. Limited tank availability in our experiment precluded the use of replicates throughout the experiment. However, there were times when several tanks had received the same treatments and were, therefore, acting as replicates. This was particularly true up to the time of the second injection when there were effectively two control groups (tanks 2+3), four IPN infected groups (tanks 5, 6, 7+8) and one PD group (tank 1). After the second injection two groups of IPN-positive fish were infected with PD (tanks 6+7), two groups were positive for IPN only (tanks 5+8 except for the terminal sample) and tank 3 was, to a large extent, a repeat for tank 1 which examined the effect of PD on growth. No tank effects were detected and all groups of fish performed similarly up to the time of the first injection. Therefore, there is good evidence that the eight tanks used in the experiment, which included automated feeders and water flow meters, were providing similar environmental conditions.

The present study is the first report of experimental transmission of PD in salmon of 300 g and 400 g mean weight at temperatures of 6.5°C and 8°C respectively. The time course for the development of PD in fish from tank 3 was similar to that described by Raynard and Houghton (1993) for post-smolt salmon at temperatures between 13°C and 15°C. Fish infected with pancreas disease suffered severe growth depression sufficient to cause long term reductions in fish weight. The depression in fish growth rates observed in tank 3 coincided with the development of exocrine pancreas pathology used to diagnose PD. Evidence linking histological diagnosis of PD with reduced growth was also obtained for fish in tank 1, although confirmation that fish in tank 1 were affected by PD was only

made at the end of the experiment when growth rates had recovered. Therefore, on two occasions, histological diagnosis of PD was closely associated with reduced growth of salmon. Since the only known variable between control and PD infected populations was the origin of the material injected, we conclude that PD was the cause of the reduced growth. PD caused reduced growth rates over a period of 54 days (tank 1) which resulted in a lower mean weight equivalent to a loss of approximately 20 days growth. Although PD affected fish recovered, as evidenced by a return to normal growth rates, the lower weight was maintained for the duration of the experiment. As far as we are aware this is the first report of weight loss induced by experimentally transmitted pancreas disease and confirms reports from field studies on fish farms which have described close associations between reduced growth and pancreas disease.

IPN virus caused early pathological signs of IPN infection such as the vacuolation and shrunken appearance of a few diffusely distributed pancreatic acinar cells which were observed 15 days post-infection. The observation of shrunken and rounded pancreatic acinar cells at day 55 and 69 post-infection together with five out of 29 fish showing some true acinar cell necrosis at day 89 (tank 5) indicates that an active but low level IPN infection persisted throughout the experiment which resulted in moderate virus titres in both kidney and pancreas.

As far as we are aware, this is the first time a critical growth experiment has been carried out with IPN virus in individually marked salmon in sea water. There was no evidence that IPN affected the growth of salmon used in our study. This result may not be surprising considering the development of only slight pathology to the pancreas. The weight loss observed in the fish affected by PD is thought to relate to the absence of digestive enzymes as a result of the total loss of pancreas acinar cells (Pringle *et al.*, 1992) although loss of appetite may also be important (McVicar, 1987). Therefore, IPN may only affect the growth of fish when sufficient pancreatic acinar cells are necrotic leading to reduced levels of pancreatic digestive enzymes. The development of such a severe pathology may require specific conditions allowing IPN virus to be pathogenic.

There are many possible explanations for the low pathogenicity of IPN virus in our study. The pathological effect of an intra-peritoneal injection of IPN virus may be dose dependent. Perhaps higher doses of virus or a method of infection more closely resembling the natural route of entry of virus into fish would produce greater pathological changes. The size and age of fish and time following transfer to sea water may also affect susceptibility to IPN. Rimstad *et al.* (1991) reported that doses of $10^{4.5}$ g^{-1} fish in 110 g post-smolts produced no clinical effects and no pathological changes. Smail (unpublished data) using smaller (55 g) post-smolts found that intra-peritoneal injection at doses between 10^4 and 10^6 g^{-1} fish at 12°C produced marked pancreas pathology and recoverable virus in the pancreas and kidney. Attenuation of the Sh strain of IPN should not have occurred since only three passages of the isolate had been made before injection and Hill and Dixon (1977) found that IPN strain Sp remained pathogenic for rainbow trout fry following up to five passages in cell culture.

The resistance of salmon in fresh water to IPN is very age dependent. Swanson and Gillespie (1979) found that yearling Atlantic salmon showed no clinical signs but various degrees of pancreas pathology when infected with IPN whereas younger fish were more susceptible. Perhaps the resistance to IPN which is developed in yearling salmon in fresh water is retained by salmon of post-smolt age and greater. Salmon in sea water may only become susceptible and develop severe pathology and clinical symptoms when they are

badly affected by some other factor which increases susceptibility to IPN virus. The growth rates and weights recorded for the controls used in our study indicate that the salmon were well adapted to sea water and performing well when injected with IPN virus. Additionally, the titres of IPN virus achieved in our study were relatively low indicating that the fish were able to limit virus replication, whereas in field situations virus levels of up to 10^8 pfu.g^{-1} fish have been recorded (Smail, unpublished data).

No synergistic effect between IPN and PD was observed. On the contrary, previous infection with IPN reduced the impact of PD with fewer fish suffering weight loss and some evidence that the exocrine pancreas pathology was less severe in fish which were first infected with IPN before they were infected with PD. This ameliorative effect may have been due to non-specific defence mechanisms which had been stimulated by the prior IPN infection. Alternatively, IPN virus may have interfered with the replication of the putative PD infectious agent by, for example, blocking binding to sites within cells.

A further interaction between PD and IPN was the finding that IPN-positive fish infected with pancreas disease had lower titres of IPN virus in the pancreas/caeca. A possible explanation for this observation was that PD and IPN virus infected acinar cells were expelled from the pancreas as PD affected fish show an apparent loss of acinar cells. The positive correlation between growth rate and pancreas/caeca IPN virus titre in these fish supports that view since fish showing greatest weight loss are most likely to have been affected by PD.

We have indicated that if salmon of 300 g, in sea water, are maintained under the appropriate environmental conditions IPN virus does not affect short-term growth. However, the absence of an effect by IPN on the growth of salmon in sea water does not preclude the possibility of such an effect under conditions which are different to those used in this experiment.

Environmental and other disease conditions on salmon farms are very variable and may influence a fishes physiology in many ways. Stressful environments, for example, high stocking density, severe weather conditions, low oxygen concentrations and social stress, are known to impair disease resistance (Wedemeyer, 1970; Sniesko, 1974; Maule *et al.*, 1989) and the general health of fish perhaps making salmon more susceptible to IPN. These deleterious environmental factors in the absence of IPN virus may have serious consequences for a fishes health with IPN infection adding to one or a combination of several problems already present.

Further experiments are required in order to determine whether there are conditions under which IPN can affect the growth of salmon in sea water. Such experiments would need to consider many factors including, the age of fish, the time following transfer to sea water, the method of infection, the manipulation of environmental conditions and interactions with other diseases.

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TABLE 1

Numbers of fish in each tank showing the sequence of injections received

Tank	Number of fish at start	First injection (day 0)	Second injection (day 42)
1	30	PD	Control
2	30	Control	Control
3	30	Control	PD
4	32	Control	Control
5	30	IPN	Control
6	30	IPN	PD
7	30	IPN	PD
8	42	IPN	Control

The weights of fish in tanks 1, 2, 3, 5, 6, 7 and 8 were measured at 89, 36 and 12 days before the first injection and at the following days after the first injection; 0, 15, 28, 42, 55, 69 and 89. Tissue samples for histology and virology were taken as follows;

Day 15. Tank 4 (five fish), Tank 8 (eight fish) sampled for liver, kidney and pancreas histology plus kidney and pancreas/caeca IPN virus titre.

Day 28. Tank 4 (five fish), Tank 8 (eight fish) sampled for liver, kidney and pancreas/caeca IPN virus titre.

Day 42. Tank 4 (five fish), Tank 8 (eight fish) sampled as day 28.

Day 55. Tank 4 (five fish), Tank 7 (10 fish), Tank 8 (eight fish) sampled as day 15. Tank 3 (10 fish) sampled for histology of pancreas, liver and kidney.

Day 69. Tank 4 (five fish), Tank 7 (eight fish), Tank 8 (six fish), Tank 3 (eight fish). Sampled as for day 55.

Day 89. Tank 1 (28 fish) sampled for pancreas histology.
Tank 2 (10 fish) sampled for pancreas histology.
Tank 3 (10 fish) sampled for pancreas histology.
Tank 4 (three fish) sampled as for day 15.
Tank 5 (29 fish) sampled as for day 15.
Tank 6 (29 fish) sampled as for day 15.
Tank 7 (10 fish) sampled as for day 15.

TABLE 2

Summary of the histological effects of IPN and PD in the pancreas of salmon

Tank conditions	Time after first injection					
	15 days	28 days	42 days	55 days	69 days	89 days
Tank 4 Control (C) + C	ACL = 0 Zymogen = 5 (n=4)	No sample available	No sample available	ACL = 0 (n=5) Zymogen = 5	ACL = 0 (n=5) Zymogen = 5(n=3), 4(n=1), 3(n=1)	ACL = 0 (n=3) Zymogen = 5
Tank 8 IPN + C	ACL = 0 (n=8) Some acinar cells with vacuoles and shrunken	No sample available	No sample available	ACL = 0 (n=8) A few rounded cells	ACL = 0 (n=6) a few rounded cells Zymogen = 1(n=1), 2(n=2), 4(n=2), 5(n=1)	No sample taken
Tank 3 C + PD				ACL = 0 (n=8) ACL = 0 (n=1) ACL = 2 (n=1) Zymogen = 5	ACL = 2(n-2), 3-4(n=2), 4(n=2) Zmogen = 5	ACL = 0-1(n=1), 2(n=1), 3(n=2), 3-4(n=1), 4(n=5) Zymogen = 0(n=5), 2(n=2), 4(n=1), 5(n=2)
Tank 7 IPN + PD				ACL = 0 (n=7) 0-1 (n=3) Zymogen = 5	ACL = 2(n=4), 3-4(n=2), 2(n=4) Zymogen = 5(n=7), 2(n=1)	ACL = 0-1(n=1), 2(n=3), 2-3(n=1), 3(n=1), 3-4(n=3), 4(n-1) Zymogen = 4(n=2), 5(n=8)
Tank 6 IPN + PD						ACL = 0(n=5), 0-1(n=4), 1-2(n=2), 2(n=4), 3(n=4), 3-4(n=5), 4(n=6) Zymogen = 0(n=4), 2(n=1), 3(n=4), 4(n=5), 5(n=15)
Tank 5 IPN + C						ACL = 0(n=29) Some shrunken and necrotic cells (n=5) Zymogen = 5(n=29)
Tank 1 PD + C						ACL = 0(n=10), 0-1(n=2), 1(n=6), 2(n=4), 3(n=5), 3-4(n=1) Zymogen = 0(n=1), 2(n=4), 3(n=2), 4(n=10), 5(n=10)
Tank 2 C + C						ACL = 0(n=10) Zymogen = 3(n=1), 4(n=2)

Key

ACL = Acinar cell loss: 0 = no or a few cells affected; 1 = 25% loss; 2 = 50% loss; 3 = 75% loss; 4 = 100% loss
Zymogen level (approximation), 0 = no zymogen present in any unaffected acinar cells. 5 = maximum level
Liver and Kidney: All livers and kidneys examined appeared within the range for the controls and were within the normal range for Atlantic salmon

Figure 1. The effect of IPN and PD on the growth rate of Atlantic salmon

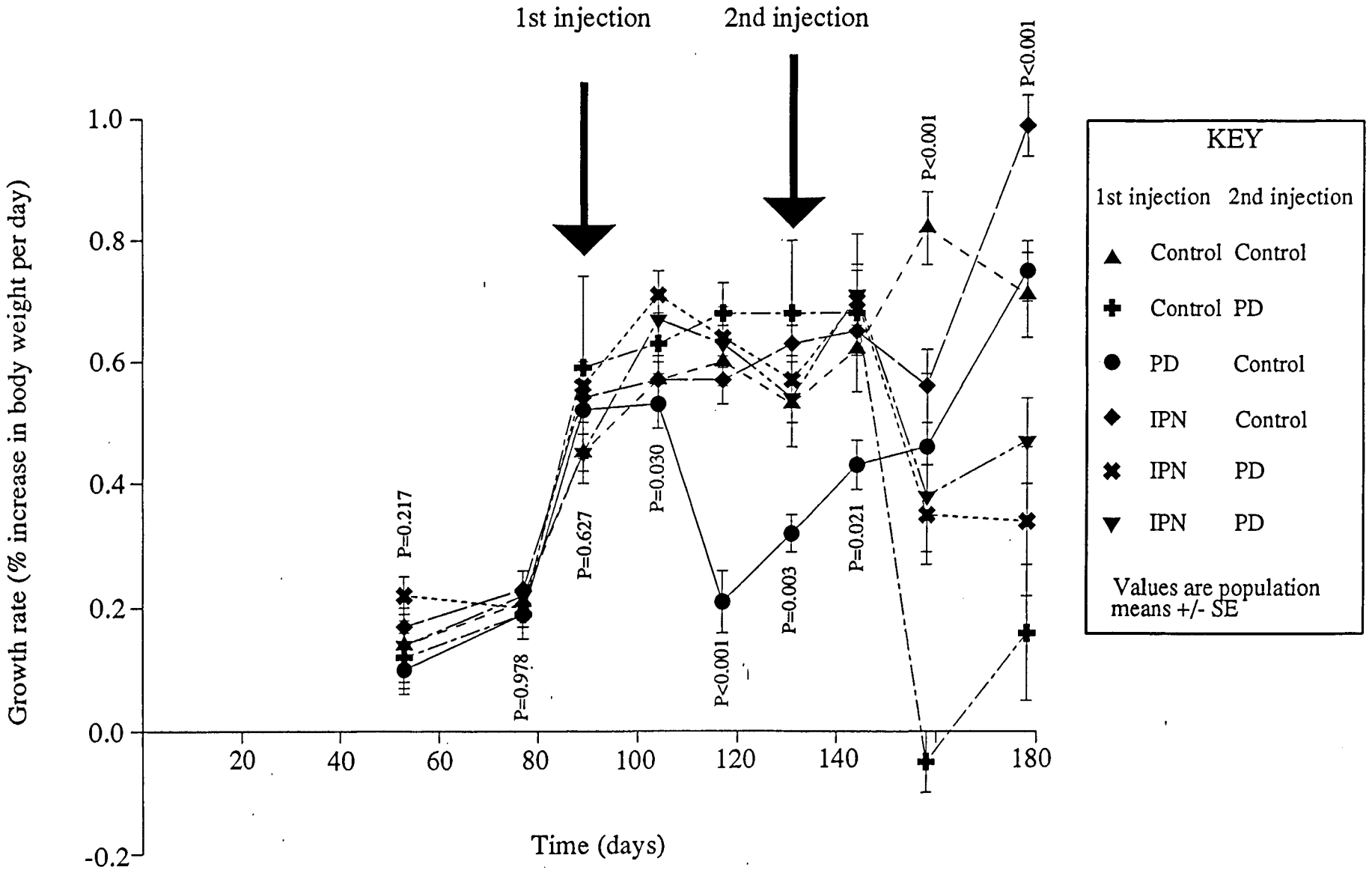

Figure 2. The effect of IPN and PD on the weight of Atlantic salmon.

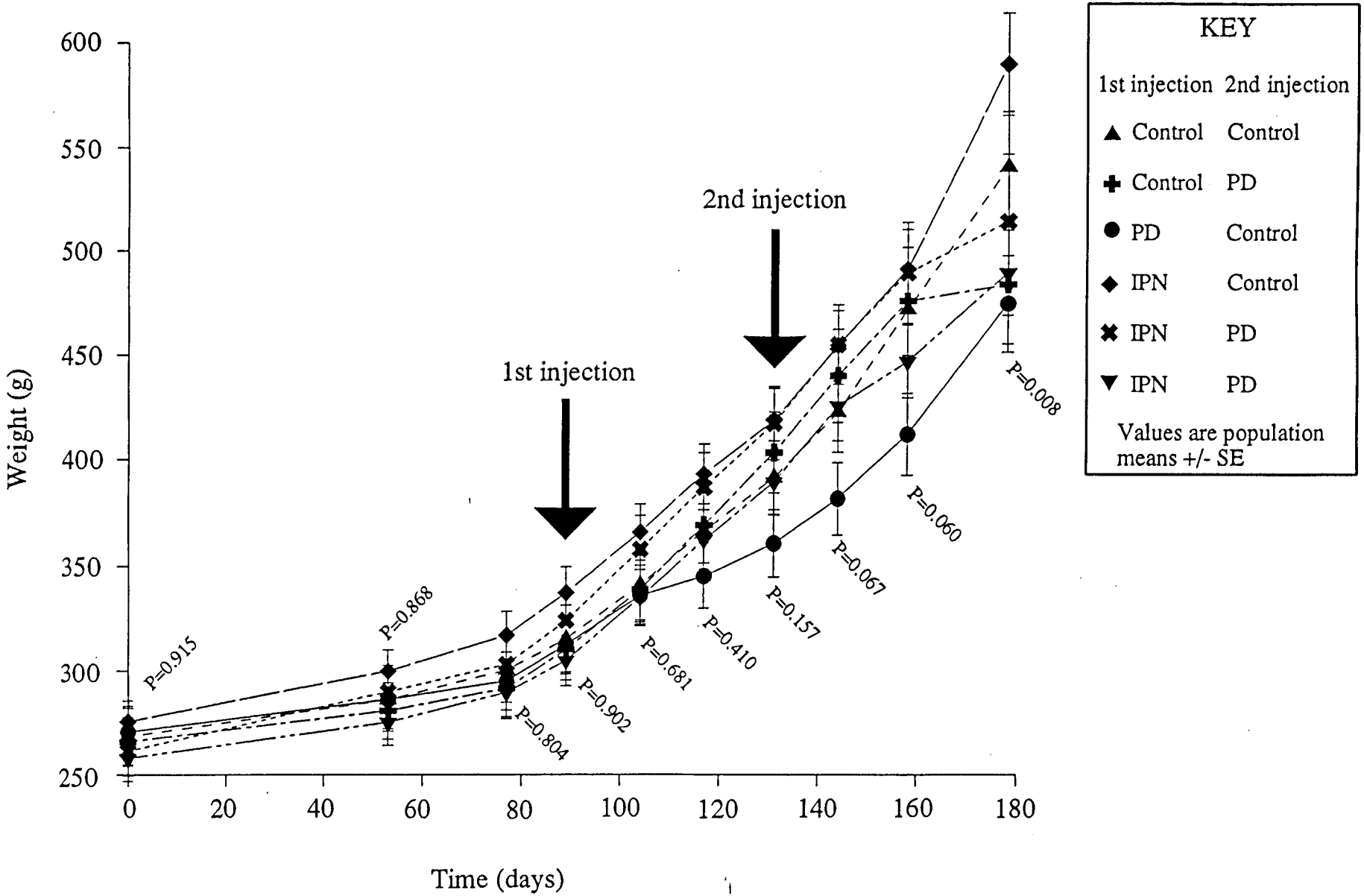

Figure 3. IPN virus titre from Atlantic salmon kidney versus time in the presence and absence of PD

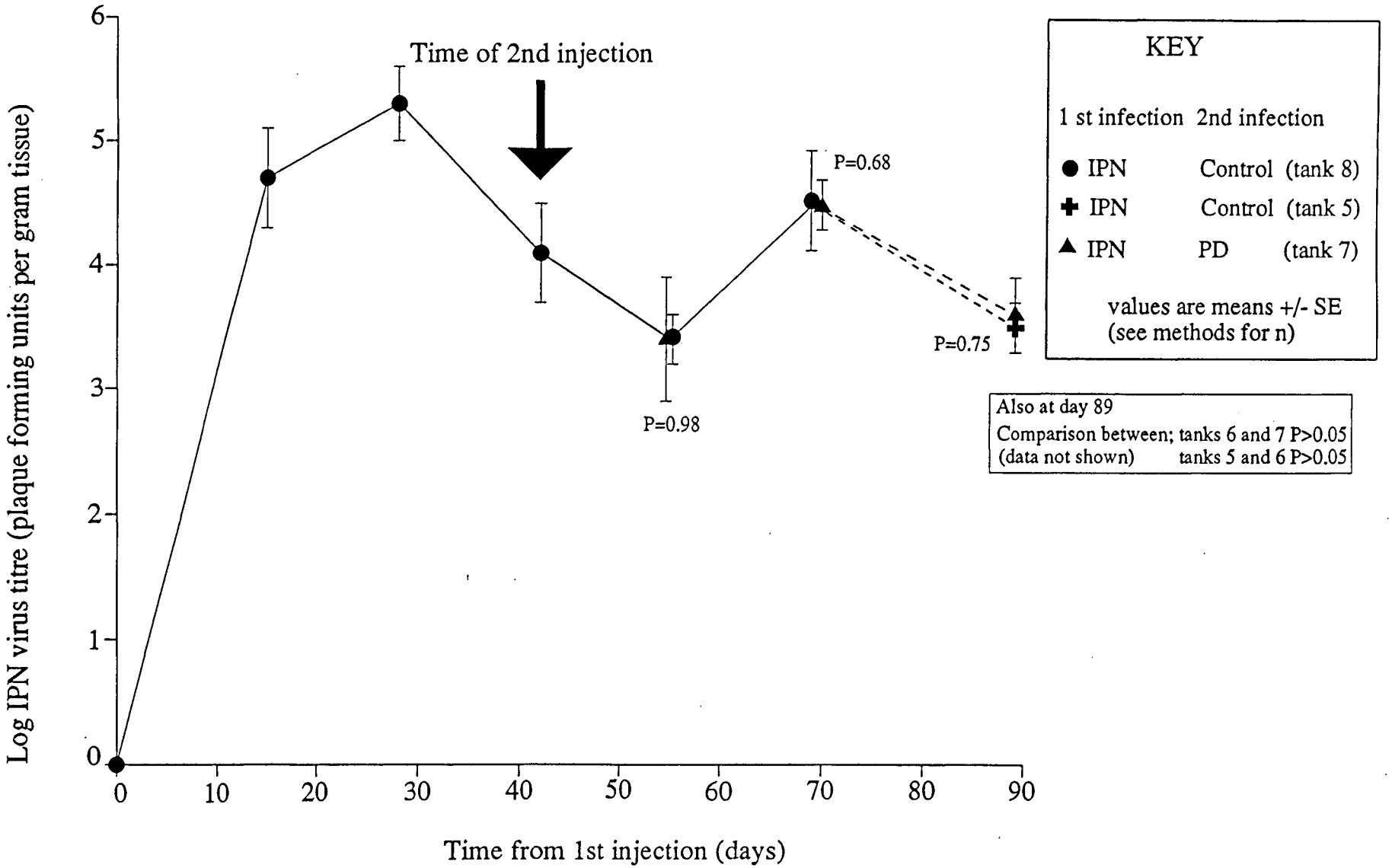

Figure 4. IPN virus titre from Atlantic salmon pancreas/caeca versus time in the presence and absence of PD

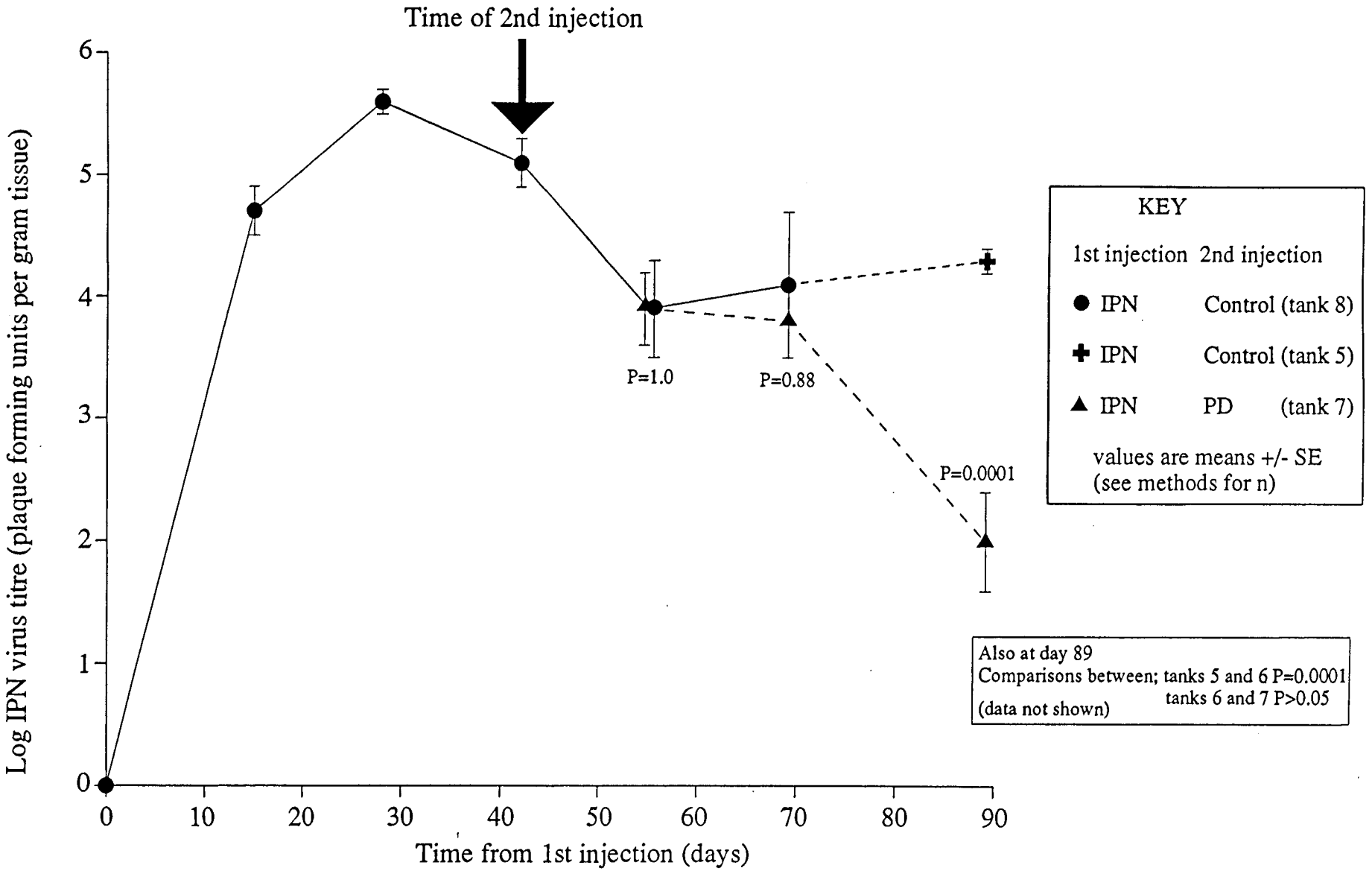

Figure 5. Plot of pancreas/caeca IPN virus titre versus growth rate in Atlantic salmon. Measurements were made 89 days after infection with IPN and 45 days after infection with PD (tank 6, $P<0.01$) a positive correlation was also found for fish from tank 7, $P<0.05$ n=10