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A PHYSIOLOGICAL EXAMINATION OF SMOLTIFICATION IN WILD SEA TROUT (*SALMO TRUTTA* L.) IN IRELAND: Na^+/K^+ -ATPase ACTIVITY, SUCCINIC DEHYDROGENASE ACTIVITY, METABOLIC ACTIVITY AND SEA WATER TOLERANCE.

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Abstract.

Wild sea trout were sampled at regular intervals between February and June 1994. Subgroups of trout from each sample period were either sampled from freshwater, subjected to a 24 hour sea water challenge test, or were monitored in a fish respirometer. Wild sea trout underwent a process of smoltification prior to their anadromous migration from fresh to sea water, showing significant changes in their sea water tolerance, gill and intestinal Na^+/K^+-ATPase activity and gill succinic dehydrogenase activity. Upon entry to sea water, trout showed a further maturation of their hypo-osmoregulatory apparatus. Sea trout smolts, however, exhibited a poorer defined parr-smolt transformation relative to Atlantic salmon as they failed to show the typical decrease in body condition and increase in total or cellular metabolic rates, during smolting. These results are discussed in terms of their significance to fisheries and aquaculture management of sea trout in Ireland.

Introduction.

Salmonids undergo behavioural, morphological and physiological changes, termed smoltification, prior to their anadromous migration from fresh to sea water (see reviews by Hoar, 1976, 1988; Folmar and Dickhoff 1980; Wedemeyer *et al.*, 1980; McKeown, 1984; McCormick and Saunders 1987). The principal physiological mechanisms associated with smoltification have been extensively studied in Atlantic and Pacific salmon. As a result, the hatchery rearing conditions necessary for producing functional Atlantic and Pacific salmon smolts have for the most part been elucidated. Such studies have indicated that initiation of smoltification is influenced by photo-period, temperature and lunar phase. These abiotic factors are thought to stimulate the alteration in production, release and sensitivity by target organs to a suite of hormones, principally, thyroid hormone, growth hormone, prolactin, cortisol, arginine vasotocin, natriuretic peptides. These hormones may act individually or synergistically to influence a variety of biochemical processes. The principal processes associated with the parr-smolt transformation include, a reduction of total body lipids, increased total metabolic activity, changes in gill, kidney and intestinal Na^+/K^+ adenosine triphosphatase (ATPase) activity, increased gill succinic dehydrogenase activity, increased intestinal absorption rates, decreased glomerular filtration rates and decreased immuno-competence. Ultimately, the highly synchronized biochemical changes result in an increased hypo-osmoregulatory ability by migrating smolts that enables them to survive and grow in sea water.

By contrast, the process of smoltification in sea trout (*Salmo trutta* L.) has been less extensively researched. The aim of the present study was to investigate the process of smoltification in wild sea trout under normal conditions. A more comprehensive understanding of smoltification in sea trout may further enhance rearing techniques employed in hatcheries involved in the programme

to restock fisheries in western Ireland affected by the stock collapses between 1989 and 1994 (Anon. 1995).

Materials and Methods.

Samples of trout and salmon were electro-fished from the Brusna River, (Moy tributary) on February 9, March 15 and April 21 1994 to collect parr, pre-smolt and smolt stages of development, respectively. Fish were transported to the aquarium in University College Dublin and were allowed to acclimate for three days in tanks supplied with de-chlorinated freshwater. Thereafter, a proportion of the cohort was subjected to a standard 24 hour sea water challenge test; fish maintained in freshwater acted as controls. The four or five trout remaining were placed into a freshwater respirometer (Fig. 1). The oxygen consumption of the fish was monitored over a three day period after an initial acclimation period of 4 days in the respirometer.

All fish were sacrificed by cranial concussion, blood was removed from the caudal vessels and blood plasma was obtained by centrifugation. Data on body weight, colouration (subjectively assigned a score of 1-3 for colour of dorsal and ventral surfaces, presence/absence of red spots, parr marks and caudal tip darkening), fork length and sex were collected for each fish. Plasma osmolality was measured using a Gonotec Osmomat® 030 automatic cryoscopic osmometer. Gill, kidney and intestinal ATPase activity were analyzed using a modification of Zaugg's (1982), Henkel *et al.* (1988) and Mayer-Gostan and LeMaire's (1991) assay techniques. Gill succinic dehydrogenase (SDH) activity was measured using a modification of method of Langdon and Thorpe (1985).

Statistics.

One-way ANOVA and Student-Newman-Keuls (SNK) multiple range tests were used to assess for significant differences in plasma osmolality, gill succinic dehydrogenase activity, gill, kidney and intestinal Na^+/K^+-ATPase activity, over time. The relationships between the enzymatic activity of Na^+/K^+-ATPase activity in the gills, kidneys and intestine with that of hypo-osmoregulatory ability and gill succinic dehydrogenase activity were tested by least squared linear regression. The body colouration scores were analyzed using principle component analysis with the aim of representing the five parameters as one independent variable.

Results.

Trout examined from the Brusna River exhibited a significant change (One-way ANOVA $P<0.001$) in gill ATPase activity over the period of the study, February to June 1994. Gill ATPase activity remained low among the February and March groups and increased significantly (SNK test $P<0.05$) in the sea trout smolts caught on April 21 (Fig. 2). These smolts had a significantly elevated gill ATPase activity compared to brown trout sampled at the same time (SNK test $P<0.05$). A comparison of the profile of salmon and trout from the Brusna River showed that gill ATPase activity remained elevated for longer in trout than salmon when retained in freshwater after the smolt run. The average values of the peaks in activity were slightly higher in salmon than sea trout smolts.

The trout in the study displayed a clear increase in their ability to hypo-osmoregulate between parr, pre-smolt and smolt stages of development. Only 5% of the trout captured in February survived 24 hours exposure to sea water and these survivors were osmoregulating poorly. The survival and ability to osmoregulate in sea water increased in the March sample group (Table 1). A significantly (SNK $P<0.05$) enhanced ability to tolerate salt water was demonstrated by the sea

trout smolts sampled in April. The close association between the ability of fish to osmoregulate and their gill ATPase activity can be shown by the significant linear relationship between both parameters ($P<0.001$, $r^2=0.63$).

The intestinal ATPase activity of trout changed significantly over the sample period (One-way ANOVA $P<0.001$). The activity of the enzyme elevated significantly among the April 21 and 30 sample groups (SNK $P<0.05$) and decrease significantly in the June sample to a pre-smolt level (Fig. 3). There was a significant linear correlation between gill and intestinal ATPase activity from trout examined in 1994 ($P<0.001$, $r^2=0.47$). By contrast, there was no apparent relationship between the activity of ATPase in the kidneys with that in the gills or intestine. There was no apparent change in kidney ATPase over the study period (data not shown).

The activity of gill succinic dehydrogenase activity (SDH) remained relatively constant between February and May (Fig. 4). The activity increased significantly in the June 18 sample (SNK test $P<0.05$). The transfer of sea trout smolts to sea water resulted in a stimulation in gill succinic dehydrogenase activity (Fig.6). The activity of the enzyme increased significantly after 56 days exposure to sea water (SNK test $P<0.05$) and remained elevated thereafter. A similar significant ($P<0.05$) stimulation in gill ATPase activity by exposure to sea water was observed in trout acclimated to salt water for 56 days (Fig. 5). The coincident elevations in the activity of both enzymes in the gills are demonstrated by the significant linear relationship between gill ATPase and SDH activity when all trout sampled in 1994 are pooled for the analysis ($P<0.001$, $r^2=0.48$).

An examination of the total metabolic activity of trout indicated a slight increase in activity between the February and March groups (Fig. 9). Thereafter, the metabolic activity remained relatively constant without any significant changes.

The first principal component (PC1) from the principal component analysis accounted for nearly 70% of the variance between the five body colour parameters. Trout with a PC1 <-0.5 were classified as resident trout or parr, while fully silvered smolts typically had a PC1 of >0.5 (Fig. 7). Intermediate PC1 scores represented the pre-smolt stage of development. Figure 7 shows a clear progression in body silvering by trout over the period February to April. Potential smolts could not be distinguished from resident parr until the March 15 sample (Fig. 7). Trout with an elevated gill ATPase activity exhibited a high degree of silvering with PC1 scores typically above 0.5. However, a proportion of the trout that were fully silvered had relatively low gill ATPase activity, resulting in the curvi-linear relationship shown in Figure 8.

There was no significant change in body condition of trout during the sample period (data not shown). Similarly, there was no apparent difference in body condition between sea trout smolts and resident parr sampled in April.

Table 1. Percentage survival and hypo-osmoregulatory ability of wild trout subjected to 24 hour sea water challenge tests.

Date collected	*Osmolality Mean*±SEM	*Mortality rate (%) rate after 24 hours*
16/2/94	50.1 ± 0.6	95
21/3/94	44.6 ± 0.8	50
24/4/94	26.9 ± 1.9	3

Osmolality is expressed as the mean and standard error of the percentage increase in plasma osmolality of sea water challenged trout above the mean of the freshwater control group.

Discussion.

The results of the present study indicate that wild trout can undergo a process of smoltification confirming previous observations on hatchery reared trout (Boeuf and Harache, 1984; Hogstrand and Haux, 1985; Sovio *et al.*, 1989; Tanguy *et al.*, 1994). Wild trout sampled from the Brusna River exhibited an obvious peak in ability to survive and hypo-osmoregulate in sea water, during the period of the normal smolt run (April).

A coincident peak in the ability to osmoregulate was observed in the activity of the enzyme ATPase in the gills and intestine of trout. The significant linear relationship between gill ATPase activity and hypo-osmoregulatory ability is evidence of the pivotal role this enzyme plays in the ability of fish to tolerate sea water. The close association between gill ATPase activity and smoltification has been documented for sea trout (Boeuf and Harache, 1984; Fleming, 1983; Tanguy 1993) and a variety of other salmonid species (see review by Boeuf, 1993). A comparison of the temporal profile of branchial ATPase in trout and salmon indicated that the peak in activity of the enzyme was more acute in the later species. This confirms the observations of Aydemirova et al. (1990), Muona and Soivio (1992) and Tanguy *et al.* (1994) that smoltification is not as clear nor complete in sea trout compared to salmon. The ability by trout to maintain elevated gill ATPase activity for longer than salmon may explain why the duration of the sea trout smolt run is often more prolonged than for Atlantic salmon smolts (LeCren, 1985; Anon., 1993).

Studies on metabolic activity of Atlantic salmon during smolting indicated an increase in total oxygen consumption (Higgins 1985; Maxime *et al*, 1989), and activity of succinic dehydrogenase activity in the liver (Blake *et al.*, 1984) and gills (Langdon and Thorpe, 1985; Chernisky, 1986) in smolts compared to parr. Conversely, there was no apparent increase in total metabolic activity or gill succinic dehydrogenase activity in the wild trout during the smoltification period February to April. This may represent a lack of commitment by sea trout smolts to undergo the drastic alterations in metabolic activity which result in loss of body lipid reserves and may explain why sea trout smolts failed to exhibit the decrease in body condition (Tanguy *et al.*, 1994; present study) typical of smoltifying salmonids.

Although sea trout undergo a physiological transformation prior to marine entry, they showed a further increase in gill and intestinal ATPase activity and gill succinic dehydrogenase activity after exposure to sea water for 56 days. Exposure times of only two weeks in sea water were not sufficient to stimulate an increase in gill ATPase activity (Anon. 1995). This period of maturation of the hypo-osmoregulatory apparatus may represent a vulnerable epoch in the life cycle of sea trout.

Examination of kidney ATPase in the present study showed that the activity of this enzyme was a poor indicator of smoltification status. Other studies on kidney Na^+/K^+ATPase activity and associated changes in urine production during smoltification have only been examined in a few species. Morphological studies have revealed that the reduction in glomerular filtration rate and urine production during adaptation of euryhaline fish from fresh to sea water (Holmes and Stainer, 1966) is achieved through a reduction the area of glomeruli, the number of renal corpuscles and the number of functional glomeruli in the kidney (Corville, 1983; Hwang and Wu, 1988). Associated with the reduced kidney function in sea water adapted fish is a reduction in kidney Na^+/K^+-ATPase activity in killifish (Epstein *et al.*, 1969). However, Jampol and Epstein (1970) found no such change in kidney Na^+/K^+-ATPase activity in the American eel after transfer to sea water. While, McCarthney (1976) found a slight decrease in kidney Na^+/K^+-ATPase

activity of smolting Atlantic salmon. Therefore, the role that kidney ATPase plays in smoltification and transition to sea water remains equivocal.

Body colouration is an obvious indicator of smolting in salmonids and displays a clear development in silvering during smolting in the wild trout examined. However, comparison of body colouration with gill ATPase activity indicated that colouration is not a reliable indicator of smolt status. Some fully silvered fish exhibited branchial ATPase activity typical of resident trout. The caveat that body silvering is not a reliable indicator of smolt status and sea water tolerance has been emphasized by a number of authors (see review by Langdon, 19**). Therefore, to ensure a successful transfer of reared sea trout smolts from fresh to sea water a proportion of the cohort should be either subjected to a standard 24 hour sea water challenge test or their branchial ATPase activity analyzed, prior to transfer.

To summarize, wild sea trout undergo a process of smoltification prior to their anadromous migration from fresh to sea water. Upon entry to sea water, they undergo a further maturation of their hypo-osmoregulatory apparatus. Sea trout smolts, however, exhibit a poorer defined parr-smolt transformation relative to Atlantic salmon, as they failed to show the typical decrease in body condition probably as a result of the lack of an increase in total or cellular metabolic rates, during smolting.

Acknowledgments

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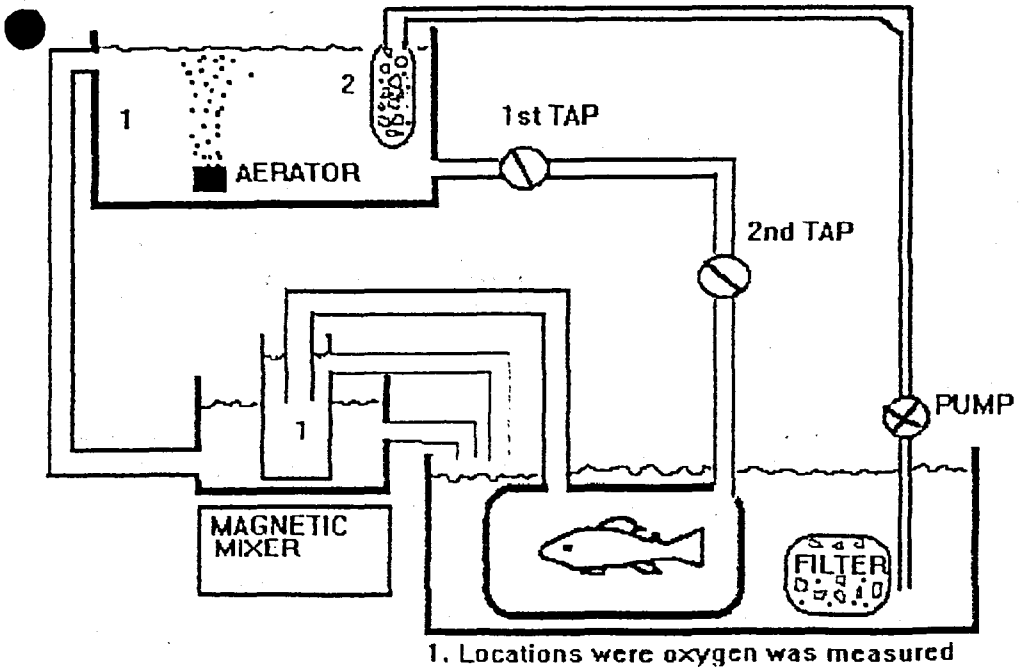
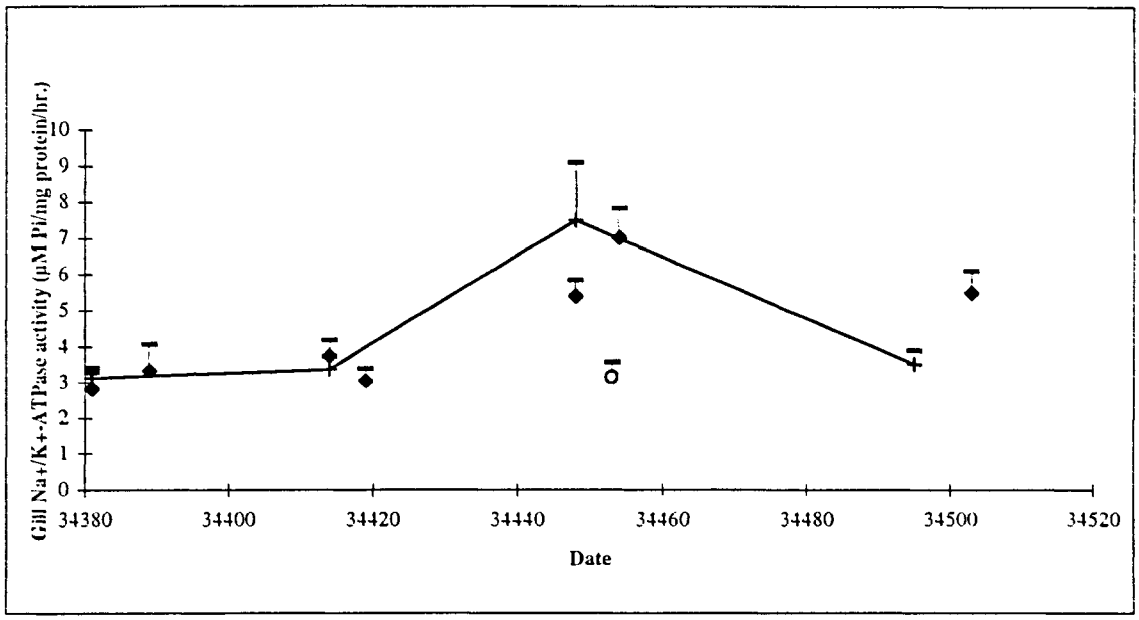


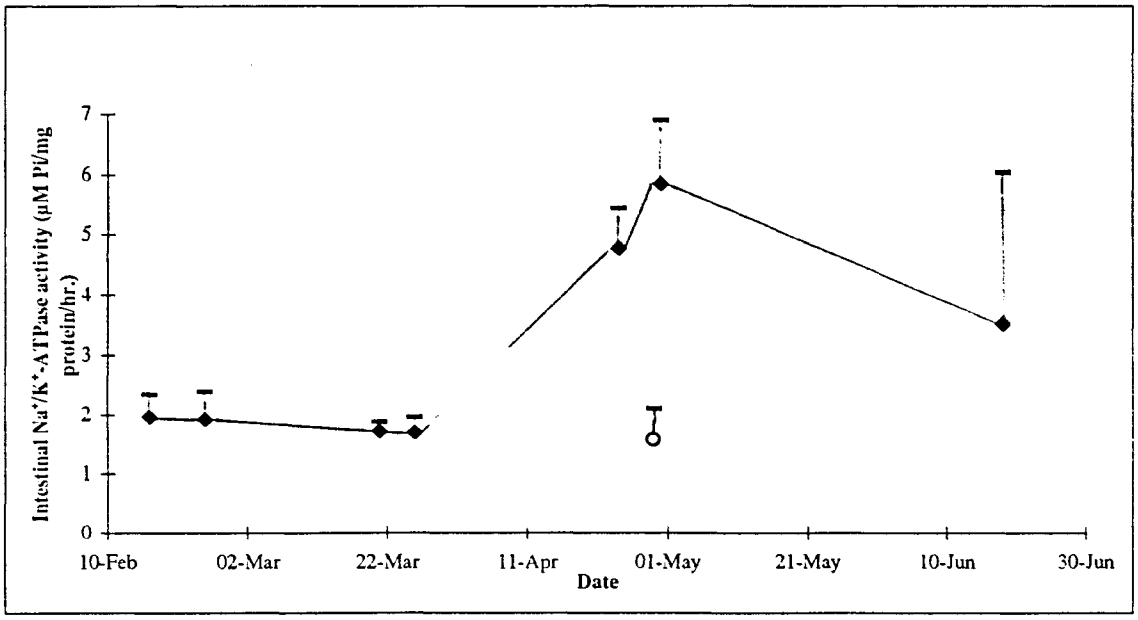
Figure 1. Schematic diagram of the fish respirometer

Figure 2. Temporal profile of gill Na^+/K^+-ATPase activity of wild trout and salmon sampled in 1994



Mean and standard error of the mean (SEM) of gill ATPase activity of trout (closed diamonds) and salmon (crosses with intersecting lines). Resident trout are distingiushed by an open circle in the April sample.

Figure 3. Temporal profile of intestinal Na^+/K^+-ATPase activity of wild trout.



Resident trout are distinguished by an open circle in the April sample.

Figure 4. Temporal profile of gill succinic dehydrogenase activity of wild trout.

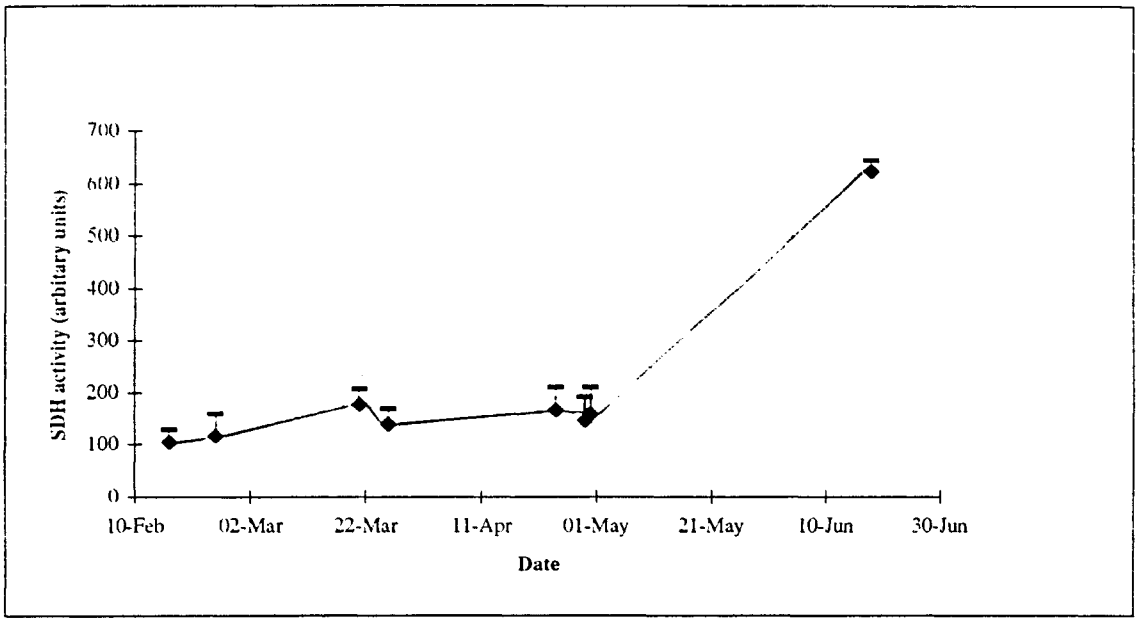


Figure 5. Influence of sea water exposure on gill Na^+/K^+-ATPase activity in wild trout.

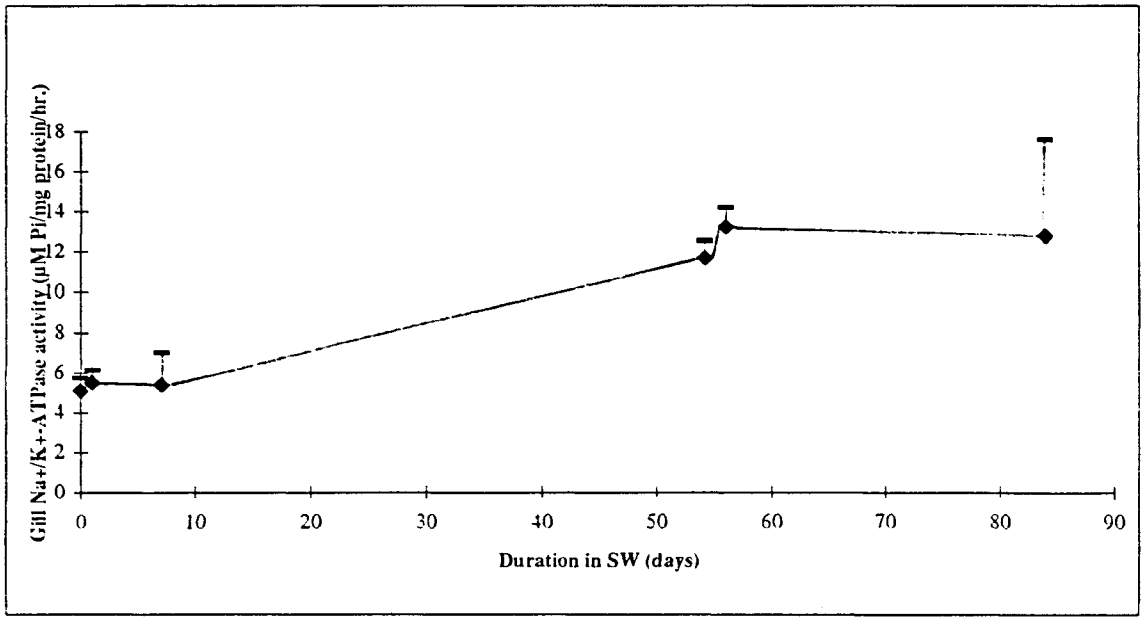


Figure 6. Influence of sea water exposure on gill succinic dehydrogenase activity in wild trout.

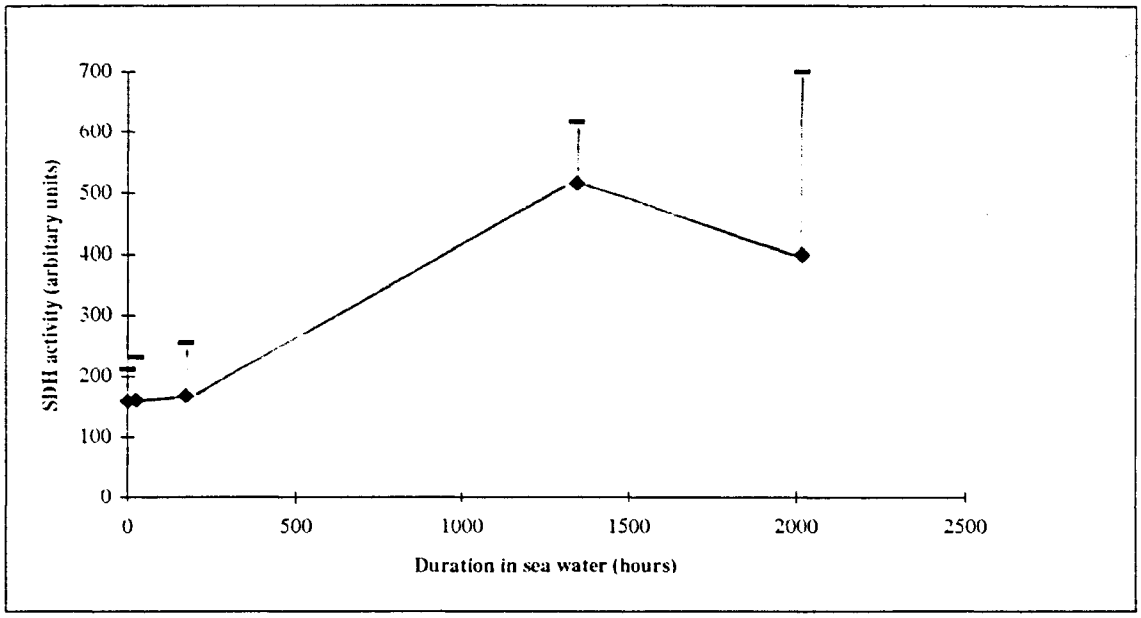
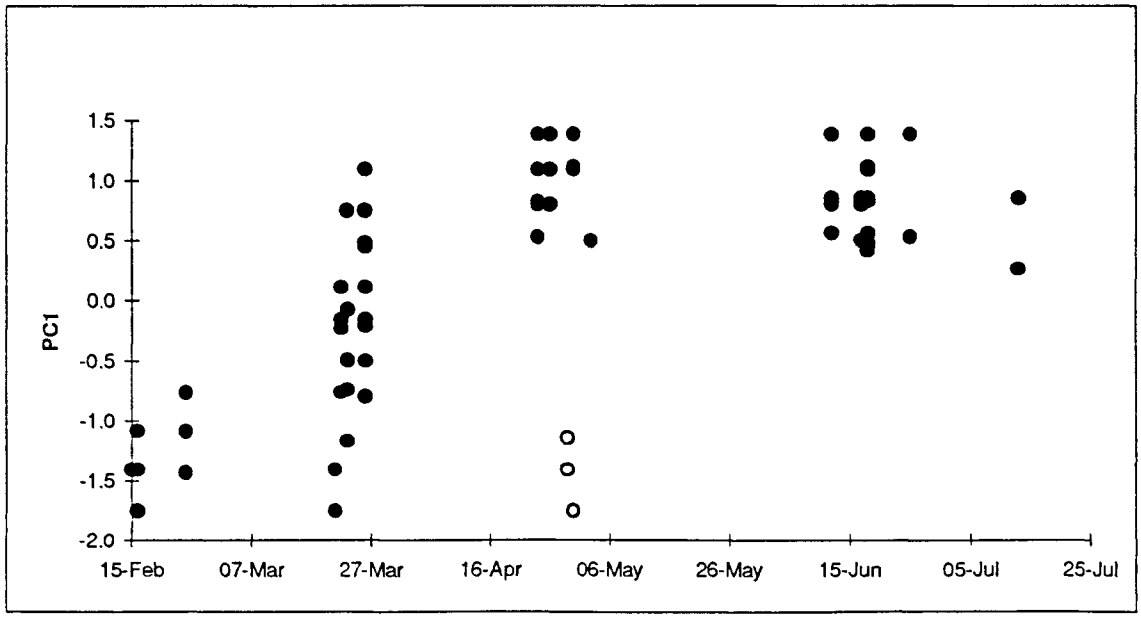


Figure 7. Temporal profile of body colouration of wild trout.



PC1 represents the first principle component from a principle component analysis of five body colour parameters measured. Resident trout are distinguished by open circles in April.

Figure 8. Relationship between body colouration and gill Na^+/K^+-ATPase activity in wild trout.

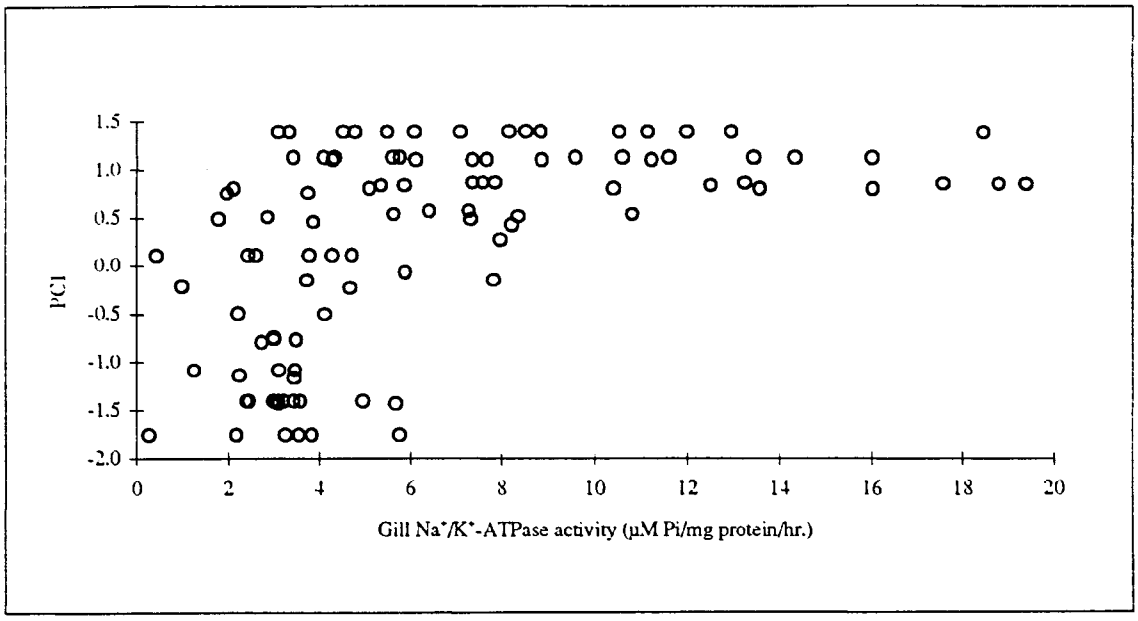
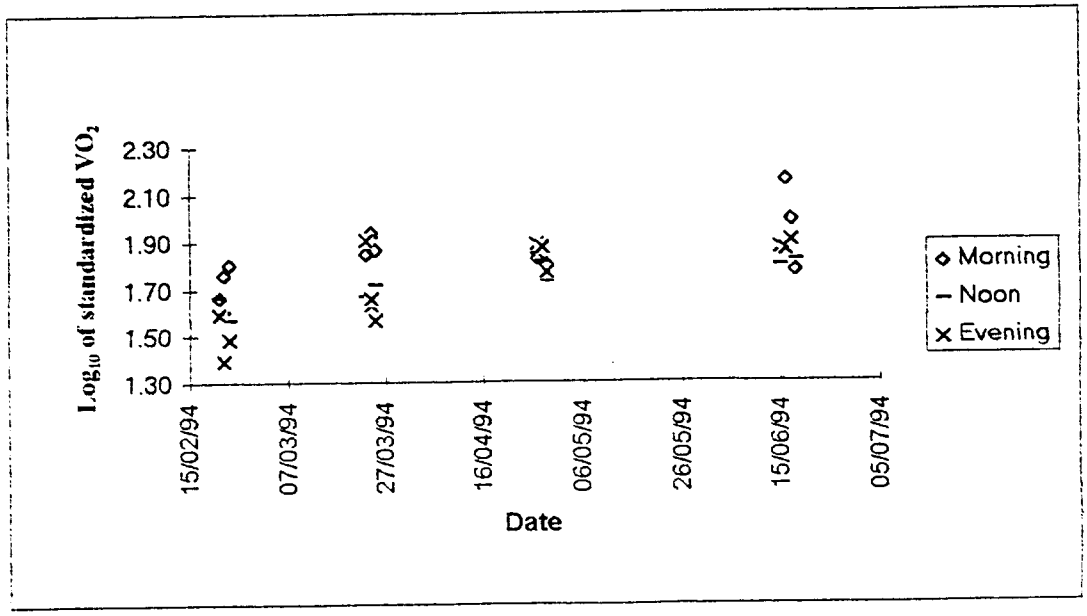


Figure 9. Temporal profile of total metabolic activity of wild trout.



The metabolic rate is expressed as $\log_{10}$ of the oxygen consumption (mg O_2/kg fish/hour) standardised for weight assuming an allometric relationship of 0.67 for weight and metabolic activity. The time of day that VO_2 was measured is distinguished by morning, noon and evening.