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Fish Welfare Research Programme

Effects of Retention of Fish in Keepnets

Institute of Freshwater Ecology

R&D Technical Report W8

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Effects of Retention of Fish in Keepnets

T.G. Pottinger

Research Contractor:
Institute of Freshwater Ecology

Environment Agency
Rio House
Waterside Drive
Aztec West
Almondsbury
Bristol
BS12 4UD

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Publishing Organisations

Environment Agency
Rio House
Waterside Drive
Aztec West
Almondsbury
Bristol BS12 4UD

Tel: 01454 624400
Fax: 01454 624409

National Federation of Anglers
Eggington Junction
Derbyshire
DE65 6GU

Tel: 01283 734735
Fax: 01283 734799

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This report will be used to provide the EA and the NFA information on the retention of fish in keepnets with respect to stress. The information will be used to initiate further research into this area of study.

Research contractor
This document was produced under R&D Contract 511 by:

The Institute of Freshwater Ecology
Windermere Laboratory
The Ferry House
Far Sawrey, Ambleside
Cumbria LA22 0LP

Tel: 015394 42468 Fax: 015394 46914

Project Managers
The Environment Agency's Project Managers for R&D Contract 511 were:
S. Rowden - Head Office (Bristol), A. Taylor - Anglian Region

The National Federation of Anglers Project Manager for R&D Contract 511 was:
Dr P. Bottomley

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EXECUTIVE SUMMARY

Capture by angling, and subsequent confinement within keepnets, exposes fish to several adverse factors. These primarily comprise the potential for physical damage arising from capture and handling, exposure to potentially harmful changes in water quality within the keepnets, and physiological stress resulting from the combination of stimuli experienced by the fish during and subsequent to capture.

Concern within the angling and fisheries management community regarding the welfare of fish populations subject to angling pressure has led to research on this subject. The question of water quality within keepnets during the confinement of fish has been addressed in a previous study by the Institute of Freshwater Ecology, comissioned by the National Federation of Anglers (Pottinger, 1992). This study established that statistically significant changes in water quality do occur during the confinement of fish within keepnets but that the magnitude of these changes does not represent a threat to the well-being of the fish.

The aim of the present study was to address a second area of concern related to angling practices; that of physiological stress associated with the capture, handling, and confinement of fish within keepnets.

All forms of disturbance evoke a stress response in fish, and under certain circumstances the stress response can have damaging effects on growth, reproductive performance, and the immune system. In order to assess the impact of angling practices on fish populations, it is therefore important to establish the extent to which capture and confinement of fish within keepnets causes physiological stress.

The present study employed two approaches to quantify the degree of stress experienced by captured and confined fish. In the first, a population of carp (*Cyprinus carpio*) maintained within an experimental facility, under controlled conditions, was employed. Groups of carp were removed from their holding tanks by net, briefly handled and allowed to struggle in a small tank to simulate manipulations associated with capture by angling, and either immediately returned to the holding tank, or transferred to a keepnet for four hours before being released into their holding tank. Blood samples were removed from the carp at intervals during this procedure and levels of three parameters which are substantially altered by stress; plasma cortisol, glucose and lactate, were determined. Plasma cortisol levels are widely employed as a direct measure of the degree of stress experienced by an animal. Cortisol is a steroid hormone and levels of cortisol, and other closely related corticosteroids, are rapidly elevated following the onset of stress in all animal groups, including fish. Cortisol is a key element in the "stress response" of fish and the magnitude and duration of its increase is proportional to the severity of the stressful stimulus. Glucose is rapidly mobilised under stressful conditions, largely under the influence of the catecholamine hormones, and measurement of changes in plasma glucose levels provides information on the extent of the metabolic response to the stressful stimulus. Lactate is an endpoint of anaerobic metabolism and increases in blood lactate levels provides evidence for extreme physical exertion, which exceeds the aerobic

capacity of the fish. The second experimental procedure employed a population of carp maintained under semi-natural conditions within a large earth lagoon. Batches of carp were captured from the lagoon by angling and transferred to keepnets. Blood samples were taken at intervals following capture and levels of cortisol, glucose and lactate were again determined.

Capture of carp from tanks by netting or from semi-natural conditions by rod and line, was found to elicit changes in the indices of stress measured. Substantial increases in plasma cortisol, an index of the primary stress response, were observed in captured carp, compared to undisturbed carp. Transfer of carp to keepnets immediately after capture, and their retention therein for four hours, did not increase, or reduce, the magnitude of the stress response when compared to that observed in carp captured and immediately released. The duration of the cortisol response to capture, with or without subsequent confinement was limited to between 4 and 24 hours after the onset of disturbance. There was no evidence of chronic elevation of plasma cortisol levels. The post-capture plasma cortisol elevation was accompanied in some cases by disturbances in plasma glucose and lactate levels but, where such changes occurred, they were limited to between 4 hours and 48 hours after the onset of the procedure.

These data suggest that the retention of carp in keepnets following capture may not represent an additional source of stress, over and above that of capture, and may not affect the rate of recovery of the fish from the initial capture stress. Capture and confinement of fish for periods of up to four hours appears to constitute an acute, rather than chronic, stress and as such is unlikely to have an adverse long-term effect on the well-being of the fish. This conclusion is based on the *physiological* changes observed in the fish, and does not take into account the possible additional effects of physical damage incurred during capture, unhooking and confinement.

Considering the physiological implications of these data alone and taking into account the experimental conditions under which they were obtained, there appears to be little basis for restricting the use of keepnets by anglers.

Further research should examine the universality of these conclusions with regard to species of fish, size of fish, water temperature, and within-keepnet stocking densities.

It is also important that further research is directed at determining the extent to which physical damage occurs during capture and unhooking, and transfer to, confinement within, and release from, keepnets. This aspect of angling procedure represents the period during which the fish is most vulnerable.

KEY WORDS: Carp, keepnets, angling, stress, physiology, cortisol, lactate, glucose.

1. INTRODUCTION

1.1 Potential Problems Associated with the Use of Keepnets by Anglers

The effects of angling practices on fish is a subject receiving increasing consideration both within the sport and from outside. Attention has focused on the welfare of fish and the possibility of adverse effects resulting from their capture, both in terms of effects on individual fish and effects of angling pressure on populations of fish. Areas of concern which are highlighted include damage incurred during playing and landing the fish, hook removal, general handling effects following capture, and the retention of fish within keepnets before weighing/photographing the catch.

The use of keepnets is perceived as a particular aspect of angling which is vulnerable to criticism on several grounds. Confinement in keepnets may adversely affect fish in several ways:

1. Physical damage during transfer to and from the keepnet. This is likely to take the form of epidermal abrasion/erosion, the removal of the fishes protective mucus layer, and scale loss.

2. Exposure of fish within keepnets to harmful deterioration in water quality. This may arise from crowding effects, if the net is overloaded, or because the net is placed in an area with a water flow which is insufficient to flush waste products from the vicinity of the net and replenish oxygen levels.

3. Initiation of a physiological stress response in the confined fish as a result of the combined effects of capture, handling, restraint and confinement.

Until recently, there were no reliable data concerning any of these specific aspects of fish welfare. However, in 1991 the National Federation of Anglers (NFA) commissioned a study to examine the nature and magnitude of changes in water quality which occur during the confinement of fish (carp) within keepnets (Pottinger 1992). This study concluded that although water quality can deteriorate significantly during the confinement of fish, resulting in reduced levels of dissolved oxygen and increased levels of ammonia and carbon dioxide, the changes do not exceed the limits of tolerance of freshwater fish. This was found to be true even with substantially above-average loadings of fish and even when micromesh (2mm mesh diameter) nets were employed.

Consequent to a joint initiative by the NFA and National Rivers Authority (NRA), the aim of the present study is to provide further information regarding possible adverse effects related to the use of keepnets, by determining the degree of physiological stress imposed on fish by a combination of capture and subsequent confinement within a keepnet. Does capture and confinement constitute an acute stress from which recovery is rapid, or does the procedure result in a prolonged response, requiring days for full recovery and leading to possible long-term adverse effects?

1.2 The Stress Response in Fish

1.2.1 The nature and role of the response to stress in fish

The maintenance of a stable internal environment (homeostasis) is a primary requirement of all organisms and is necessary to sustain life. All vertebrate animals, including fish, respond to stimuli which threaten homeostasis, or ultimately place survival of the animal in jeopardy, with a rapid adaptive physiological and behavioural response. The aim of this response is to provide the animal with the means to overcome, adapt to, or avoid, the challenge and thus restore homeostasis. This aim is achieved in part by optimising respiratory efficiency, mobilising stored energy reserves and priming the animal for extreme physical exertion (Chrousos 1992, Johnson *et al.* 1992). The response is non-specific and is similar whether the stimuli are social, physical, noxious, toxic or merely novel. This adaptive response is referred to as the *stress response* and stimuli which elicit the response are referred to as *stressors.* The application of a stressor is considered to elicit a state of *stress* in the animal. Definitions and concepts of stress in fish are discussed at length by Barton and Iwama (1991) and an overview of the stress response in fish is provided by Adams (1990), Colombo *et al.* (1990) and Wedemeyer *et al.* (1990).

In fish, as in other animals, the core of the response to stress is neuroendocrine in nature, comprising activation of the sympathetico-chromaffin system with a resultant release of catecholamines into the circulation (Gingerich and Drottar 1989) and a concomitant stimulation of the hypothalamic-pituitary-interrenal(HPI) axis; a hormonal cascade culminating in the release of the steroid hormone cortisol into the blood (Fryer and Lederis 1986, Sumpter *et al.* 1986). The neuroendocrine phase of the response is generally referred to as the *primary* stress response.

In fish, these hormones in turn act at sites within the body to elicit changes in gill blood flow, heart rate and stroke volume, the mobilisation of stored energy reserves, and alterations in the immune system (Barton 1988). These events are generally considered to comprise the *secondary* stress response.

1.2.2 Adverse effects of stress in fish

Providing that the fish is able to avoid or overcome the source of stress, the stress response fulfils its adaptive role in promoting survival. However, if the source of stress is inescapable and/or the duration of the stress is prolonged, the adaptive nature of the response is compromised and it becomes, instead, harmful. This appears to arise largely due to the fact that during the stress response, non-essential or vegetative processes, such as growth, and activity within the reproductive system, are "shut-down", and components of the immune system are suppressed. Thus, exposure of fish to prolonged, or chronic, stress causes growth suppression (Pickering 1993, Pottinger and Pickering 1992), reproductive dysfunction (Pickering *et al.* 1987, Campbell *et al.* 1994, reviewed by Donaldson 1990) and immunosuppression (Anderson 1990, Pickering and Pottinger 1989, Pottinger and Pickering 1992). Severe chronic stress will ultimately cause mortality through loss of immunocompetence and the resultant increased susceptibility to pathogenic organisms.

1.2.3 The role of cortisol as a causal factor in the damaging effects of stress

The key causal agent of the deleterious effects of chronic stress is the steroid hormone cortisol. During stress, because of the requirement for energy mobilisation, there is a switch from a state in which the acquisition and storage of energy predominates (anabolism) to a state in which stored energy reserves are broken down and mobilised (catabolism). In addition to the suppression of growth hormone observed under conditions of stress in rainbow trout (*Oncorhynchus mykiss;* Pickering *et al.* 1991) prolonged elevation of plasma cortisol levels by administration of the hormone to otherwise unstressed fish has been demonstrated to reduce growth rate in rainbow trout and channel catfish (*Ictalurus punctatus*; Barton *et al.* 1987, Davis *et al.* 1985) although the exact mechanisms involved are not yet fully understood (van der Boon *et al.* 1991).

Elevated levels of cortisol also disrupt normal functioning of the reproductive system, by reducing circulating levels of gonadal steroids, vitellogenin levels, and gonad size, and suppressing the activity of the pituitary gonadotropes in rainbow and brown trout (*Salmo trutta*; Carragher *et al.* 1989) and tilapia (*Oreochromis mossambicus*; Foo and Lam 1993). Cortisol also interferes with the hepatic synthesis of yolk protein precursor by causing a reduction in the number of liver oestradiol receptors in rainbow trout (Pottinger and Pickering 1990). Exposure of rainbow and brown trout to stress during the reproductive period ultimately results in delayed ovulation, reduced egg size, lower sperm counts and a reduction in survival of progeny (Campbell *et al.* 1992, 1994).

Perhaps the most damaging effects of stress on fish are related to the adverse effects of elevated cortisol levels on the immune system. The links between stress, elevated cortisol levels, and immunosuppression in fish have been reviewed by Schreck *et al.* (1993). Administration of cortisol to otherwise unstressed fish significantly increases mortality due to bacterial and fungal infection brown and rainbow trout (Pickering *et al.* 1989, Pickering and Pottinger 1989) and increases susceptibility to parasitic infection in rainbow trout (Woo *et al.* 1987). Numerous reports have highlighted the direct effects of cortisol in suppressing components of the immune system in fish including reductions in circulating lymphocyte numbers in brown trout (Pickering 1984) and channel catfish (Ellsaesser and Clem 1987), suppression of the production of specific antibody-secreting lymphocytes in coho salmon (*Oncorhynchus kisutch*; Maule *et al.* 1987), reductions in the mitogenic response of lymphocytes in channel catfish (Ellsaesser and Clem 1987) and suppression of immunoglobulin M levels in masu salmon (*O. masou*: Nagae *et al.* 1994).

Because cortisol is a key element in the primary stress response and levels of cortisol in the blood provide an accurate measure of the severity and duration of the stress response, and because of the pivotal role of cortisol in mediating the damaging effects of chronic stress, this steroid has become widely employed as a major index of stress in fish.

1.2.4 The taxonomic range of research on stress in fish

Most research on the impact of stress on fish, including much of that cited above, has been carried out on salmonid fish, in particular the rainbow trout, brown trout and Pacific salmon (*O. nerka,*

O. kisutch, O. keta and *O. tshawytscha*) and therefore the effect of stress is best understood in these few species. This situation has arisen largely because of the economic importance of the salmonids worldwide in both natural fisheries and aquaculture.

There is an increasing body of work which has examined the response to stress of other species of fish, including salmonids such as the Atlantic salmon (*Salmo salar*, Waring *et al.* 1992), brook trout (*Salvelinus fontinalis*, Biron and Benfey 1994) and lake trout (*Salvelinus namaycush*, Barry *et al.* 1993), and non-salmonids such as flounder (*Platichthys flesus*, Waring *et al.* 1992), golden perch (*Macquaria ambigua*, Braley and Anderson 1992, Carragher and Rees 1994), channel catfish (*Ictalurus punctatus*, Limuswam *et al.* 1983, Mazik *et al.* 1994), red drum (*Sciaenops ocellatus*, Thomas and Robertson 1991), and striped bass (*Morone saxatilis*, Young and Cech 1993a, b).

However, there has been no detailed work carried out on the physiological response to stress of those species of non-salmonid fish native to the United Kingdom and which represent the principle quarry of "coarse" anglers. Limited data are available for only four non-salmonid British fish, the common carp (*Cyprinus carpio*, Canals *et al.* 1989, Dabrowska *et al.* 1991, Jeney *et al.* 1992, Kakuta and Murachi 1992, van Dijk *et al.* 1993), the perch (*Perca fluviatilis*, Haux and Sjöbeck 1985), the roach (*Rutilus rutilus*; Vinogradov and Klerman, 1987), and the pike (*Esox lucius*, Schwalme and Mackay 1985).

The similarity of the data presented in the papers cited here suggests that the basic response to stress remains the same in fish regardless of species studied and that the response of salmonid fish to stress represents an appropriate general model. This is an unsurprising observation considering the adaptive importance of the stress response and its conservation within all vertebrate groups. However, two recent studies have presented data suggesting that the salmonid model may not be universally applicable to all species of fish. Both tilapia (Balm *et al.*, 1994) and the sea raven (*Hemitripterus americanus*; Vijayan and Moon, 1994) display responses to stress which differ in detail from that of salmonids. This suggests that some degree of caution should be employed in extrapolating data obtained from one species to another.

1.3 Keepnet Confinement and Stress: Study Aims and Methodology

The data available at present suggest that most forms of disturbance or physical challenge will initiate a stress response in fish. If the stimulus and response are short in duration (hours) no long-term adverse effects on the well-being of the fish would be expected. However, if the response is prolonged (days) evidence of the damaging effects of chronic stress, outlined above, might be expected. It is prudent to assume that the procedures and events associated with capture by angling, and confinement within keepnets, will prove to be stressful to fish. However, the question which must be addressed is; does capture and confinement constitute an acute stress from which recovery is rapid, or does the procedure result in a prolonged response, requiring days for full recovery and leading to possible long-term adverse effects?

The intention of the present study was to determine the severity (acute or chronic) of the stress imposed on fish by their retention within keepnets. In this respect, three possible effects of keepnet confinement can be envisaged. Confinement may constitute a severe stress, recovery

from which is prolonged, extending over many days, thus causing adverse long-term effects symptomatic of the exposure of fish to chronic stress. Alternatively, confinement may represent an acute stress, hours in duration, from which recovery is rapid leading to no long-term problems. Finally, it is possible that keepnet confinement is not perceived by the fish as being stressful and no effects are observed.

1.3.1 Experimental procedure

A basic experimental design was adopted for the project in which groups of carp were subjected to keepnet confinement for a period of four hours, equivalent to the duration of a typical angling match. The degree of stress experienced by the fish was assessed by quantifying components of the primary and secondary stress response. Fish are never exposed to keepnet confinement without the additional disturbance associated with capture, therefore confinement of the experimental fish was preceded by a period of physical disturbance intended to simulate capture. In order to establish whether keepnet confinement had any additive effects over and above those of capture, keepnet-confined fish were compared with groups of fish which were subjected to simulated capture but immediately released. A third group of fish, which remained undisturbed throughout the experimental period, was employed to provide evidence of baseline variability in the parameters studied.

To provide a comparison between the effects of the experimental procedure (simulated capture with and without confinement) and the effects of confinement following capture by rod and line, an experiment was carried out in which rod-caught carp were immediately transferred to keepnets and monitored for a similar period.

1.3.2 Determinands

As described in Section 1.2.3, measurement of plasma cortisol has become the standard method of obtaining a reliable and sensitive index of the severity and duration of stress in fish. Cortisol was therefore selected as an appropriate index of the primary stress response for the present study.

Plasma glucose levels were monitored to provide information on the behaviour of a catecholamine/cortisoldependent index of the secondary stress response and to provide valuable complementary information regarding the severity and duration of the response, and the time required for recovery from the stimulus. Determination of plasma glucose also provides another point of reference with previous work on the same and other species.

Plasma lactate levels were also determined to provide evidence of the extent of respiratory disruption imposed by the experimental treatments. Elevated blood lactate levels arise through anaerobic respiratory activity in which glycogen stores are depleted and lactate accumulates in the muscle tissue. Thus lactate is not considered to be an indicator of stress *per se* but rather reflects the occurrence of exhaustive exercise. Lactate was measured during the present study to establish that the procedure employed to mimic capture and handling in Experiments 1 to 3 provided comparable physiological disturbance to that observed in rod-caught carp during Experiment 4, and to determine the time required for recovery from the respiratory effects of handling.

1.3.3 Species of fish employed

Ideally, the species of fish employed for a study of the physiological effects of keepnet confinement should reflect those which are most commonly exposed to keepnet confinement. In the United Kingdom this encompasses species such as roach, dace or bream. However, there are several drawbacks associated with the use of such species.

1.A study of this nature requires that supporting data are available from previously published studies to lend validation to the levels of determinands measured in unstressed and stressed fish. As noted in Section 1.2.4 substantial data are available for only one non-salmonid British freshwater fish, the common carp, *Cyprinus carpio*. No studies have been carried out which characterise the endocrine stress response of other UK coarse fish. To undertake the study using a species for which supporting data are unavailable would require that the response of the selected species to stress be fully characterised before undertaking the experiments. This would have led to substantially larger project.

2. The aim of this study, as noted in Section 1.1, is to examine the *physiological* response of fish to keepnet confinement, independent of other factors such as possible physical damage incurred during the capture and confinement procedure. Therefore, it is appropriate that a species widely perceived to be physically robust is employed such that damage, if incurred during handling, is minimal and does not contribute to the stress arising from the procedures under examination. The possible effects of physical damage arising from capture and confinement of fish is a separate issue and should be addressed in an independent study.

3. The study requires maintenance of multiple holding tanks of fish to permit the replication necessary to accomodate the time-point samples. The population of fish must be fully acclimated to the holding conditions prior to the start of the study. The IFE facility, which has been purpose-designed to accomodate experiments of this nature using salmonid fish has not, in the past, proved suitable for maintaining non-salmonid fish, other than carp.

For these reasons, the study was carried out using common carp.

2. METHOD

2.1 Fish

Common carp (*Cyprinus carpio* L.) were purchased from Humberside Fisheries (Cleaves Farm, Skerne, Driffield, Yorkshire) in March 1991 and February 1994. The carp were maintained within the IFE experimental fish facility in partially covered 1500 l circular glassfibre tanks, each supplied with a constant flow (10 l min^{-1}) of lake water at ambient temperature. The fish were fed 5 times weekly on commercial feed (BP Mainstream) *ad libitum*. Stocking densities did not exceed 0.5 kg l^{-1} inflow. All experimental manipulations were carried out in the same tank system.

2.2 Experimental Procedure

Four experiments were carried out. An initial "range-finding" experiment to establish the time-course of changes in the determinands following capture and confinement (Experiment 1), two time-course studies employing small and large carp respectively (Experiments 2 and 3), and a capture and confinement experiment employing rod-caught carp under semi-natural conditions (Experiment 4).

2.2.1 Experiment 1

Aim: A "range-finding" study to determine the time-course of changes in the determinands following capture and confinement of carp, prior to the design of the definitive experiments

The experimental procedure is summarised in Table 2.1. In February 1994 one hundred and twenty common carp (weight at time of experiment 568 ± 22 g; length 27.6 ± 0.3 cm; n = 30, mean ± SE) were distributed evenly between three holding tanks (A, B, C) and allowed to acclimate for three weeks. Water temperature during the experiment was 4°C. At time 0 five fish were netted from tank B into a bucket containing anaesthetic (2-phenoxyethanol, 1:2000, Sigma). Blood samples were removed from the caudal vessels of each fish into heparinized syringes and transferred to polypropylene tubes. These were placed on ice. The fish were returned to a recovery tank and took no further part in the procedure.

The 35 fish remaining in tank B were netted into a 50 l glassfibre trough containing water and after five minutes were returned to their holding tank (group designated "netted"). The crowding, physical exertion and aerial exposure associated with this procedure were intended to mimic the nature of disturbances associated with capture by rod and line.

Five fish were removed from tank C and blood sampled. The remaining 35 fish were transferred to a 50 l trough and after 5 minutes were placed in two keepnets suspended in the holding tank (group designated "keepnet confined"). The keepnets employed conformed to the requirements of the local Fisheries By-Laws (Keenets K34 Round, 6mm Fre-Flo mesh, 2.5 m x 40 cm).

Five fish were also sampled from tank A but the 35 fish remaining in the holding tank were not disturbed further (group designated "controls").

One hour after the original disturbance, five further fish were removed from tank A, anaesthetised and blood-sampled. Five fish were also remove from the nets suspended in tank B and from the otherwise undisturbed tank C. Four hours after the original disturbance a further five fish were removed from each group and the remaining keepnet-confined fish (tank B) were released back into their holding tank. Further blood samples were taken 24, 48 and 168 hours after the start of the experiment.

Blood samples were spun down in a refrigerated bench-top centrifuge and plasma was aspirated and frozen at -20°C in labelled tubes until required for assay.

Table 2.1 A schematic representation of Experiment 1

<table>
<tr><th>Time of blood sampling[1]</th><th>Tank A (control)</th><th>Tank B (netted)</th><th>Tank C (keepnet-confined)</th></tr>
<tr><td>0 h</td><td colspan="3">Five fish netted from each tank into anaesthetic, blood sampled and transferred to a recovery tank</td></tr>
<tr><td rowspan="5">1 h
4 h

24 h
48 h
168 h</td><td rowspan="5">Remaining 35 fish in holding tank undisturbed</td><td>Remaining 35 fish transferred for 5 mins to 50 l trough to mimic capture and handling.</td><td>Remaining 35 fish transferred for 5 mins to 50 l trough to mimic capture and handling.</td></tr>
<tr><td rowspan="4">All fish returned to holding tank</td><td rowspan="2">All fish transferred to keepnets suspended in holding tank for 4h</td></tr>
<tr></tr>
<tr><td rowspan="2">All fish released from keepnets and returned to holding tank</td></tr>
<tr></tr>
</table>

[1] At each sample point, five fish were netted with the minimum of disturbance from each tank into anaesthetic, blood-sampled, and transferred to a recovery tank.

2.2.2 Experiment 2

Aim: To determine the time-course of changes in plasma cortisol, lactate and glucose levels in small carp subjected to simulated capture alone, or capture combined with a period of keepnet confinement

The experimental procedure is summarised in Table 2.2. In February 1994, fifteen hundred common carp were divided evenly between fifteen holding tanks. Initial mortality rates within this population were high enough to preclude their immediate experimental use. Between 5 February and 1 May 890 fish were lost. No causal agent was identified. The population did not respond to antibiotics and although there was evidence of fungal infection this, or protozoan ectoparasite infestation, did not appear to be primary causes of mortality. However, from May onwards, mortality levels were negligible and the fish were feeding well. The fish were redistributed evenly among the available tanks and left undisturbed, other than routine feeding and cleaning, for 2 months prior to the experiment.

In July 1994, when the experiment was carried out, there were forty fish per tank (weight of fish at time of experiment 44.9 ± 1.9 g; length 11.6 ± 0.2 cm; n = 30, mean ± SE) and water temperature was 15°C. Sufficient numbers of fish and adequate tank space were available to avoid the repeated disturbance of individual tanks which occurred in Experiment 1 with the exception of the 168 hour sample which was taken from tanks which were sampled at 1 hour.

The fifteen tanks were designated as Controls (A1-A5), Netted (B1-B5), or Keepnet-confined (C1-C5), five tanks in each treatment group. At time 0, six fish were netted from tank 1 within each treatment group, anaesthetized and blood-sampled as for Experiment 1. The forty fish in each of the remaining Netted or Keepnet-confined tanks (four tanks of each) were netted and transferred to 50 l troughs. After five minutes, fish from the Netted group were returned to their holding tanks, while the fish from the Keepnet-confined group were transferred to keepnets suspended within their holding tanks (Keenets K34 Round, 6mm Fre-Flo mesh, 2.5 m x 40 cm). Fish in the remaining four Control tanks were not disturbed. At 4 h after the initial disturbance the fish confined within keepnets were released into their holding tanks. At 1, 4, 24, 48 and 168 h samples of six fish were taken from one Control (undisturbed) tank, one Netted tank and one Keepnet-confined tank.

Table 2.2 A schematic representation of Experiment 2

<table>
<tr><th>Time of blood sampling[1]</th><th>Tanks sampled</th><th>Tanks A1-A5 (control)</th><th>Tanks B1-B5 (netted)</th><th>Tanks C1-C5 (keepnet-confined)</th></tr>
<tr><td>0 h</td><td>A1, B1, C1</td><td colspan="3">Six fish netted from one tank in each group into anaesthetic, blood sampled and transferred to a recovery tank</td></tr>
<tr><td rowspan="6"></td><td rowspan="6"></td><td rowspan="6">Tanks A2 - A5 remain undisturbed</td><td>Fish from tanks B2 - B5 transferred for 5 mins to 50 l trough to mimic capture and handling,</td><td>Fish from tanks C2 - C5 transferred for 5 mins to 50 l trough to mimic capture and handling,</td></tr>
<tr><td rowspan="5">All fish returned to holding tanks</td><td rowspan="3">All fish transferred to keepnets suspended within holding tanks for 4h</td></tr>
<tr><td>1 h</td><td>A2, B2, C2</td></tr>
<tr><td>4 h</td><td>A3, B3, C3</td></tr>
<tr><td rowspan="3"></td><td rowspan="3">All fish released from keepnets into holding tanks</td></tr>
<tr><td>24 h
48 h
168 h</td><td>A4, B4, C4
A5, B5, C5
A2, B2, C2</td></tr>
</table>

[1] At each sample point, six fish were netted with the minimum of disturbance from the designated tank into anaesthetic, blood-sampled, and transferred to a recovery tank.

2.2.3 Experiment 3

Aim: To determine the time-course of changes in plasma cortisol, lactate and glucose levels in large carp subjected to simulated capture alone, or capture combined with a period of keepnet confinement

The experimental procedure is summarised in Table 2.3. A second time-course experiment was carried out in July 1994 employing the larger carp used in Experiment 1. The remaining large carp used for the range-finding study (weight 505 ± 30 g; length 27.4 ± 0.4 cm; n = 30, mean ± SE) were distributed evenly between twelve holding tanks, five fish per tank. The fish were allowed 4 weeks to acclimate to these conditions. The experimental procedure was identical to that for Experiment 2, except that limited numbers of fish dictated that the 24 h and 48 h samples were omitted. Five fish were sampled at each time point. Water temperature during the experiment was 15°C.

Table 2.3 A schematic representation of Experiment 3

<table>
<tr><th>Time of blood sampling[1]</th><th>Tanks sampled</th><th>Tanks A1-A4 (control)</th><th>Tanks B1-B4 (netted)</th><th>Tanks C1-C4 (keepnet-confined)</th></tr>
<tr><td>0 h</td><td>A1, B1, C1</td><td colspan="3">Five fish netted from one tank in each group into anaesthetic, blood sampled and transferred to a recovery tank</td></tr>
<tr><td rowspan="4"></td><td rowspan="4"></td><td rowspan="4">Tanks A2 - A4 remain undisturbed</td><td>Fish from tanks B2 - B4 transferred for 5 mins to 50 l trough to mimic capture and handling</td><td>Fish from tanks C2 - C4 transferred for 5 mins to 50 l trough to mimic capture and handling</td></tr>
<tr><td rowspan="3">All fish returned to holding tanks</td><td rowspan="2">All fish transferred to keepnets suspended within holding tanks for 4h</td></tr>
<tr><td>1 h</td><td>A2, B2, C2</td></tr>
<tr><td>4 h</td><td>A3, B3, C3</td><td rowspan="2">All fish released from keepnets into holding tanks</td></tr>
<tr><td>168 h</td><td>A4, B4, C4</td></tr>
</table>

2.2.4 Experiment 4

Aim: To determine the time-course of changes in plasma cortisol, lactate and glucose levels in carp captured from a semi-natural environment by rod and line and transferred *to a* keepnet.

This experiment was carried out at the National Rivers Authority (North West region) fish farm, Leyland E. T. W., Emnie Lane, Leyland, Lancs.. The facility comprises a number of purpose-built earth lagoons, 50m x 30m containing a variety of coarse fish bred on-site for re-stocking purposes. The experiment was carried out in March 1991 as part of an NFA-commissioned study on water quality within keepnets during the confinement of fish, although the data were not published as part of the project report (Pottinger, 1992).

Batches of ten common carp (mirror variety; mean weight 120 ± 15 g, n = 40) were captured from a single lagoon by two anglers in quick succession and either immediately anaesthetised and blood-sampled, or placed in keepnets (Keenets K9 and K34, 10mm and 6mm mesh, 2.5 m x 40 cm) and blood-sampled at 1, 2, 3, 4 or 5 h post-capture. Blood samples were treated as described above and plasma was stored frozen until required for assay. Water temperature was 8°C.

2.3 Assay Procedures

2.3.1 Plasma cortisol

Cortisol levels were determined by radioimmunoassay following the methodology described in Pickering *et al.* (1987). Briefly, steroids were extracted (efficiency ≥ 98%) from 200 µl of plasma with a 1.0 ml aliquot of ethyl acetate (Analar, BDH). After thorough vortex mixing, and separation of the aqueous and solvent phases by centrifugation, a 150 µl aliquot of the extract was dried under vacuum in a polypropylene assay tube, together with 20000 dpm of [1,2,6,7-^{3}H]cortisol (Amersham, 74 Ci mmol^{-1}). At the same time, a series of standard tubes was prepared, in duplicate, containing 0, 25, 50, 100, 200, 400 and 800 pg of radioinert cortisol (Sigma) together with 20000 dpm labelled cortisol in ethyl acetate. These tubes were also dried down. When both the unknown and standard tubes had been evaporated to dryness, a 200 µl aliquot of cortisol antiserum (anti-cortisol-21-hemisuccinyl-BSA, Steranti Research Ltd) diluted to working strength in 0.01 M phosphate buffer (containing 0.137M sodium chloride, pH 7.4; Sigma) was added to each tube. The tubes were vortex mixed and incubated overnight at 4°C. After incubation, a 100µl aliquot of dextran-coated charcoal suspension (0.5% activated charcoal, 0.1% dextran in phosphate-buffered saline) was added to each tube. The tubes were vortex-mixed, incubated on ice for 5 mins and spun at 1000 rpm at 4°C for 5 mins. A 200 µl aliquot of the supernatant was pipetted into a 7 ml scintillation vial and 5 ml of scintillation fluid (Ecoscint A, National Diagnostics) were added. Samples were counted under standard ^{3}H conditions in a Packard Tri-Carb 1900TR liquid scintillation counter. A standard curve was constructed of percent ^{3}H-cortisol bound versus inert cortisol present and the amount of cortisol present in the unknowns was calculated from this. Final values were expressed as ng cortisol / ml plasma.

2.3.2 Plasma glucose

Plasma glucose levels were determined by the glucose oxidase method (Sigma Diagnostics procedure no. 510). Glucose oxidase is employed to convert glucose in the sample to gluconic acid with the production of two molecules of hydrogen peroxide per molecule of gluconic acid.The hydrogen peroxide oxidises colourless o-dianisidine to a brown colour. The intensity of brown colour measured at 425-475 nm is proportional to the concentration of glucose present in the sample. A series of glucose standards (0, 5, 10, 15, 20, 25, 37.5, 50, 67.5, 75 µg/tube) was prepared in polypropylene assay tubes from a stock solution containing 50 mg glucose / 100 ml distilled water. The standards were made up to a final volume of 525 µl with distilled water. Plasma samples (25 µl) were pipetted into tubes and made up to 525 µl with distilled water. A 5.0 ml aliquot of combined enzyme and colour reagent (500 units of glucose oxidase, 100 purpogalin units of peroxidase, 4 mg o-dianisidine hydrochloride in 100 ml distilled water) was added to each tube. Tubes were vortex mixed and incubated at room temperature for 45 mins after which the absorbance of each sample at 475 nm was determined in a sipper cell spectrophotometer. A standard curve was constructed and the glucose content of the plasma samples was calculated.

2.3.3 Plasma lactate

Lactate levels were determined by an enzymatic method (Sigma Diagnostis procedure no. 735) in which lactic acid is converted to pyruvate and hydrogen peroxide by oxidase. In the presence of the hydrogen peroxide formed, peroxidase catalyses the oxidative condensation of chromogen precursors to produce a coloured dye with a absorption maximum at 540 nm. The increase in absorbance at 540 nm is proportional to the concentration of lactate in the sample. A range of standards (0, 2, 4, 6, 8, 10, 12 µg in 30 µl) was prepared in duplicate from a stock solution of lactate (40 mg/100 ml). Plasma samples (10 µl) were pipetted into assay tubes and made up to 30 µl with distilled water. To each tube, 1.0 ml of reconstituted reagent was added and tubes were mixed and incubated at room temperature for 10 mins. An additional 1.0 ml of distilled water was added to each tube and the contents of each tube were transferred to a glass cuvette. The absorbance of each sample at 540 nm was determined in a spectrophotometer. All readings were completed within 10 mins of the end of the incubation period. A standard curve was constructed and the concentration of lactate in the plasma samples calculated.

2.3.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA, Genstat) with treatment (control, netted, keepnet confined) and time (0, 1, 4, 24, 48, 168 h) as factors. The data were log-transformed where necessary, to improve homogeneity of variance.

3. RESULTS

3.1 Experiment 1

3.1.1 Plasma cortisol

Plasma cortisol levels for Experiment 1 are presented in Fig. 3.1 (a). Levels of plasma cortisol prior to the start of the experiment were slightly, but significantly ($p<0.05$) higher in the keepnet designated group (25.6 ± 7.7 ng ml^{-1}; mean ± SE, n = 5) than in the controls (10.6 ± 2.7 ng ml^{-1}) or netted (8.94 ± 3.0 ng ml^{-1}) groups. Analysis of variance of log-transformed data revealed significant effects of treatment ($p<0.001$) and time ($p<0.001$) and a significant treatment x time interaction ($p<0.001$). These effects were resolved as a significant increase in plasma cortisol levels in both netted and keepnet-confined groups relative to the controls within 1 hour of the initial disturbance. Levels in both groups were highest 4 hours after netting/confinement,reaching 271.3 ± 31.3 ng ml^{-1} in group K and 216.6 ± 31.3 $ng^{-1}ml$ in group N, both levels being significantly higher ($p<0.001$) than that in the controls (75.7 ± 10.5 ng ml^{-1}). However, within 24 hours of the initial disturbance, levels in both netted (41.7 ± 8.3 ng ml^{-1}) and keepnet-confined groups (42.6 ± 8.0 ng ml^{-1}) were statistically indistinguishable from levels in the controls (39.0 ± 7.9 ng ml^{-1}). Although there were some differences between groups at 48 hours these, although significant, were small compared to the elevations observed at 1 and 4 hours. Levels in the three groups were indistinguishable at 168 hours.

3.1.2 Plasma glucose

Plasma glucose levels for Experiment 1 are presented in Fig. 3.1 (b). There was considerable variation in plasma glucose levels within treatment groups as indicated clearly by the large standard errors associated with mean values, even in fish prior to the start of the experiment. At time 0, plasma glucose levels in the keepnet-confined group were significantly ($p<0.05$) lower than those in the control and netted groups. Overall, ANOVA did not identify a significant effect of treatment. However, there were significant effects of time ($p<0.001$) and a significant treatment x time interaction ($p<0.05$). This was resolved as a significant elevation of plasma glucose in the keepnet-confined group (146.0 ± 13.6 mg 100 ml^{-1}) relative to the control (91.4 ± 14.8 mg 100 ml^{-1}) and netted groups (105.7 ± 9.9 mg 100 ml^{-1}) at 4 and 24 hours after the onset of disturbance. There were no significant differences between the three groups at 48 or 168 hours.

3.1.3 Plasma lactate

Plasma lactate levels for Experiment 1 are presented in Fig. 3.1 (c). There were no significant differences between groups prior to the onset of disturbance. Analysis of variance of log-transformd data revealed significant treatment ($p<0.001$), time ($p<0.001$) and treatment x time ($p<0.001$) effects. These were resolved as a highly significant elevation of plasma lactate levels in both the netted (140.3 ± 25.8 mg 100 ml^{-1}) and keepnet-confined groups (126.3 ± 4.0) compared to the control group (27.3 ± 2.2) within 1 hour of the onset of disturbance. Although levels in both handled groups had declined markedly by 4 hours, they were still significantly higher than levels in the control group ($p<0.01$, $p<0.001$). There was no significant difference in lactate levels between the handled groups. At 24, 48 and 168 hours. only minor differences in

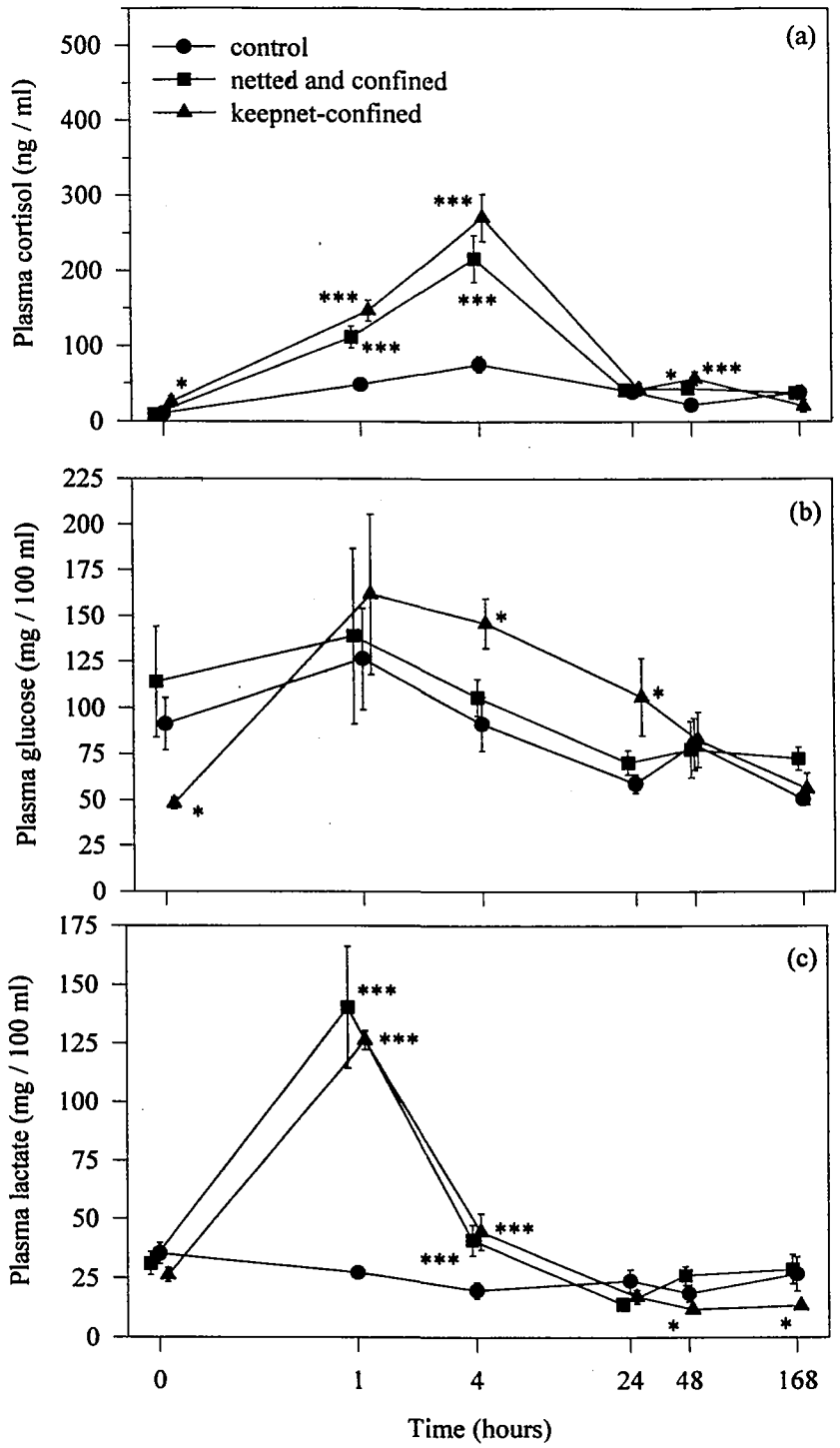

Figure 3.1. Changes in plasma cortisol, glucose, and lactate in control, netted and released, and keepnet-confined carp determined in Experiment 1. Plasma cortisol (a), plasma glucose (b) and plasma lactate (c) in undisturbed control fish (), netted and released fish (), and fish keepnet-confined for 4h (), at intervals prior to and following the onset of disturbance. Each point represents the mean ± SE, n = 5. Significant differences from control values at each time point are denoted by asterisks * $p<0.05$, *** $p<0.001$.

lactate levels were apparent.

3.2 Experiment 2

3.2.1 Plasma cortisol

Plasma cortisol levels for Experiment 2 are presented in Fig. 2.1 (a). Baseline plasma cortisol levels in undisturbed carp at time 0 in this experiment were within a similar range to those in Experiment 1 (10 - 50 ng ml^{-1}). Analysis of variance of log-transformed data revealed significant treatment, time and treatment x time effects (all $p<0.001$). These were resolved as a highly significant ($p<0.001$) elevation in plasma cortisol within 1 hour of disturbance in both netted (434.7 ± 51.8 ng ml^{-1}; n = 6) and keepnet-confined groups (448.1 ± 74.9 ng ml^{-1}) compared to the undisturbed group controls (73.5 ± 3.5). Significantly ($p<0.001$) elevated levels of plasma cortisol were maintained in both handled groups at 4 hours relative to the control group but by 24 hours after the onset of disturbance there was only a minor, though significant ($p<0.001$, 40.4 ± 5.7 ng ml^{-1}) elevation in the keepnet-confined fish compared to the netted (24.2 ± 1.9 ng ml^{-1}) and control fish (15.8 ± 4.2 ng ml^{-1}). There were no differences between groups at 48 and 168 hours.

3.2.2 Plasma glucose

Plasma glucose levels for Experiment 2 are presented in Fig. 3.2 (b). Analysis of variance revealed a significant effect of treatment ($p<0.001$), time ($p<0.001$) and a significant treatment x time interaction ($p<0.01$). These were resolved as significantly higher plasma glucose levels in keepnet-confined fish than in control or netted fish at 1 hour and 4 hours. This difference was superimposed on a general and significant ($p<0.001$) increase in glucose levels in all three groups between time 0 and 1 hour. The most pronounced difference in glucose levels was observed at 4 hours when levels in keepnet-confined fish (132.3 ± 18.5 mg 100 ml^{-1}) were markedly higher than control (64.1 ± 2.7 mg 100 ml^{-1}) or netted (77.6 ± 5.6 mg 100 ml^{-1}) fish. During the remaining samples at 24, 48 and 168 hours there were no marked differences between groups although keepnet-confined fish at 48 hours had slightly, but significantly, higher glucose levels (66.7 ± 3.8 mg 100 ml^{-1}) than control (51.3 ± 4.7 mg 100 ml^{-1}) and netted fish (55.2 ± 2.1 mg 100 ml^{-1}).

3.2.3 Plasma lactate

Plasma lactate levels for Experiment 2 are presented in Fig. 3.2 (c). Analysis of variance failed to identify a significant overall treatment effect although there were significant time ($p<0.001$) and time x treatment ($p<0.001$) effects. Lactate levels in all three groups of fish were variable for the first four samples; levels in fish in keepnet-confined fish were significantly lower at time 0 than levels in control fish (22.8 ± 3.8 cf. 37.4 ± 2.8 mg 100 ml^{-1}; $p<0.05$). Lactate levels in all three groups were higher at 1 hour than at time 0 ($p<0.001$) and were significantly lower again at 4 hours ($p<0.001$). Lactate levels in fish in the netted group were significantly lower than levels in the other two groups at 4 hours ($p<0.001$) and levels in the netted group at 24 hours were significantly higher than the control group, though not the keepnet-confined group ($p<0.001$). There were no differences between groups at 48 hours and 168 hours.

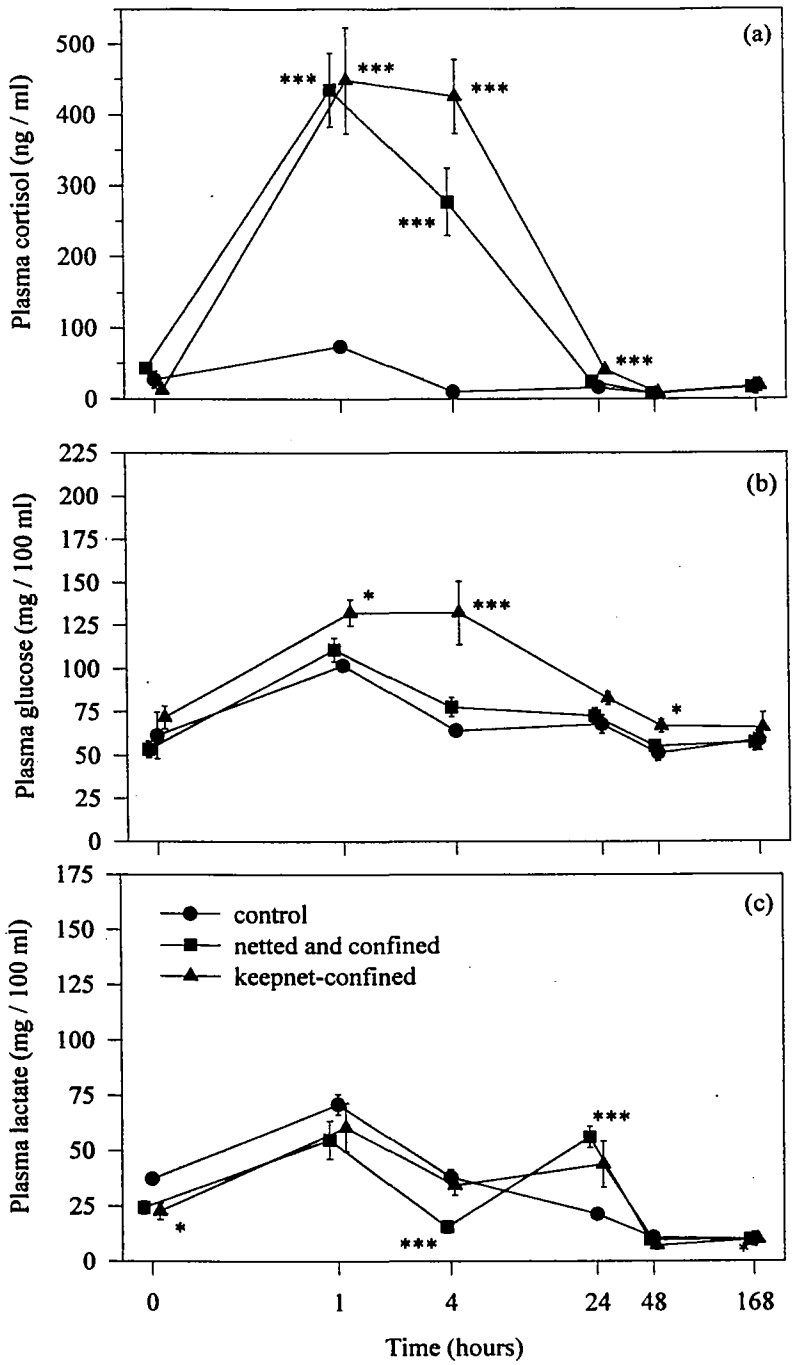

Figure 3.2 Changes in plasma cortisol, glucose, and lactate in control, netted and released, and keepnet-confined carp determined in Experiment 2. Plasma cortisol (a), plasma glucose (b) and plasma lactate (c) in undisturbed control fish (), netted and released fish (), and fish keepnet-confined for 4h (), at intervals prior to and following the onset of disturbance. Each point represents the mean ± SE, n = 6. Significant differences from control values at each time point are denoted by asterisks * $p<0.05$, *** $p<0.001$.

3.3 Experiment 3

3.3.1 Plasma cortisol

Plasma cortisol values for Experiment 3 are presented in Fig. 3.3 (a). No samples were taken at 24 and 48 hours. Analysis of variance identified significant effects of treatment, time and treatment x time interaction on plasma cortisol levels. These were resolved mainly as significantly higher levels in both handled groups at 1 hour and 4 hours. At time 0, cortisol levels in keepnet-confined fish (37.7 ± 4.4 ng ml^{-1}; n = 5) were significantly ($p<0.001$) higher than those in control (16.9 ± 4.5 ng ml^{-1}) and netted fish (8.7 ± 1.2 ng ml^{-1}). However, at 1 hour, levels in both netted (207.2 ± 44.1 ng ml^{-1}) and keepnet-confined groups (351.7 ± 86.3 ng ml^{-1}) were markedly higher than levels in the control group (6.6 ± 0.7 ng ml^{-1}; $p<0.001$) although not significantly different from each other, and although they declined, remained so at 4 hours. At 4 hours, levels in the keepnet-confined fish (125.7 ± 22.7 ng ml^{-1}) were significantly higher ($p<0.001$) than levels in both netted (37.5 ± 8.8 ng ml^{-1}) and control groups (9.6 ± 3.1 ng ml^{-1}). At 168 hours, levels in the netted group (60.5 ± 14.7) remained slightly, but significantly ($p<0.001$) higher than levels in the control (15.3 ± 3.2) and keepnet-confined groups (25.5 ± 6.2).

3.3.2 Plasma glucose

Plasma glucose levels for Experiment 3 are presented in Fig 3.3 (b). Analysis of variance revealed there to be significant effects of treatment, time, and a significant treatment x time interaction (all $p<0.001$). These effects were resolved as a significant elevation of plasma glucose levels in netted and keepnet-confined groups at 1 and 4 hours ($p<0.001$). Glucose levels in these groups rose from a baseline of 60-75 mg 100 ml^{-1} at time 0 to 112.4 ± 7.6 mg 100 ml^{-1} (netted) and 123.3 ± 9.7 mg 100 ml^{-1} (keepnet-confined) compared to a level in the controls of 56.1 ± 6.5 mg 100 ml . A slight drop in glucose levels in the handled groups was observed at 4 hours but they remained higher than control levels. No differences between the three groups were apparent at time 0 or 168 hours.

3.3.3 Plasma lactate

Plasma lactate levels for Experiment 3 are presented in Fig. 3c. Analysis of variance failed to demonstrate a treatment effect, but there were significant time ($p<0.001$) and treatment x time ($p<0.05$) effects. These effects were resolved as slightly, but significantly, higher plasma lactate levels at 1 hour in the netted group (23.9 ± 3.3 mg 100 ml^{-1}; $p<0.05$) and keepnet-confined group (33.4 ± 3.2 mg 100 ml^{-1}; $p<0.001$) than in the control group (17.8 ± 1.7 mg 100 ml^{-1}). Levels in the keepnet-confined group at 168 hour were slightly, but significantly, higher than control levels (19.1 ± 3.3 cf. 9.4 ± 2.3; $p<0.001$).

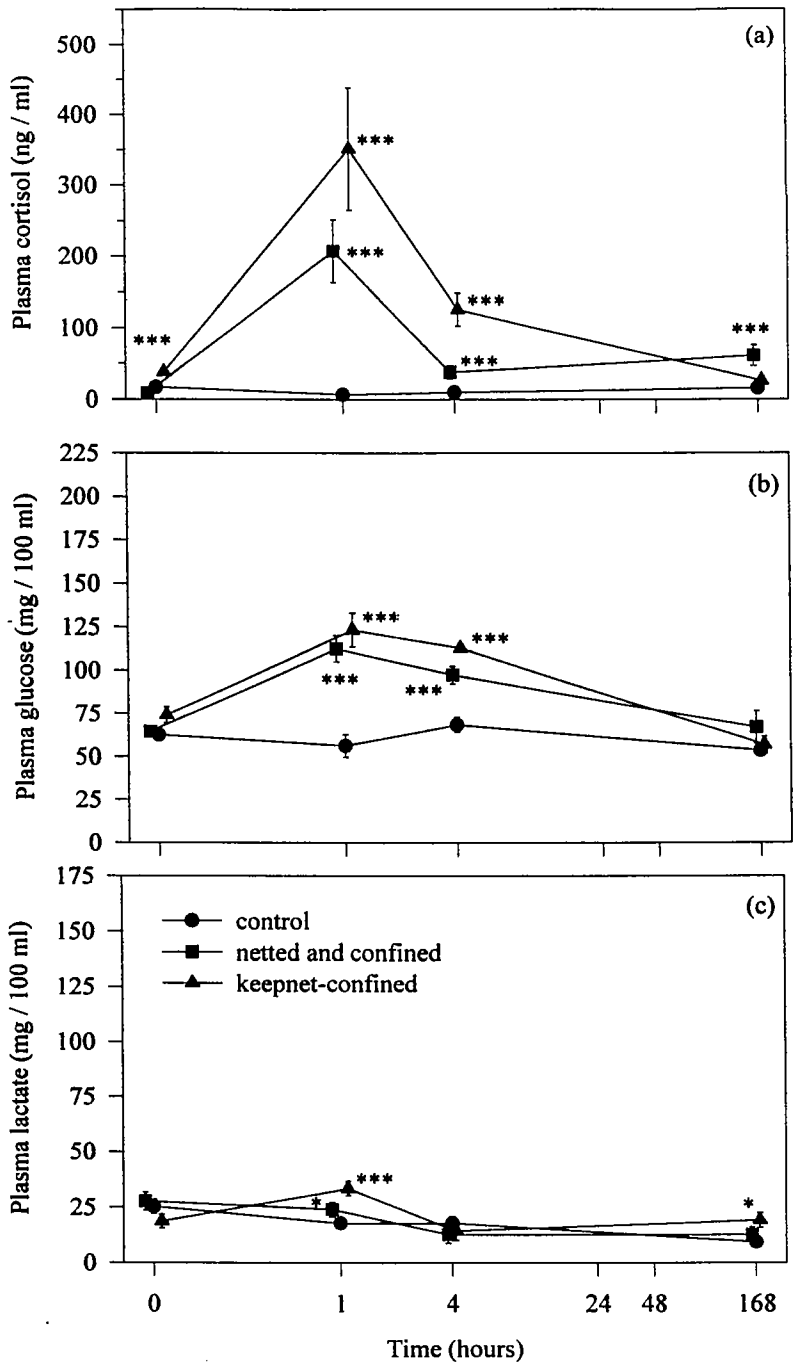

Figure 3.3 Changes in plasma cortisol, glucose, and lactate in control, netted and released, and keepnet-confined carp determined in Experiment 3. Plasma cortisol (a), plasma glucose (b) and plasma lactate (c) in undisturbed control fish (), netted and released fish (), and fish keepnet-confined for 4h (), at intervals prior to and following the onset of disturbance. Each point represents the mean ± SE, n = 5. Significant differences from control values at each time point are denoted by asterisks * $p<0.05$, *** $p<0.001$.

3.4 Experiment 4

3.4.1 Plasma cortisol

Plasma cortisol levels for experiment 4 are presented in Fig. 3.4 (a). A one-way ANOVA of log-transformed data revealed there to be a significant effect of time ($p<0.001$). This was resolved as a significant increase in cortisol levels following capture. Levels rose from a mean of 86.4 ± 19.4 ng ml^{-1} (n = 10) immediately following capture to 178 ± 18.1 ng ml after 1 hour of keepnet confinement ($p<0.01$). At the four subsequent hourly samples, cortisol levels were significantly elevated above those at time 0 at 2 hours (146.6 ± 25.1 ng ml^{-1}; $p<0.05$) and 4 hours (164.6 ± 32.3 ng ml^{-1}; $p<0.05$) but were not significantly different from 0 hour values at 3 and 5 hours after capture.

3.2.2 Plasma glucose

Plasma glucose levels for Experiment 4 are presented in Fig. 3.4 (b). Analysis of variance revealed a significant variation in glucose levels with time ($p<0.001$) which was resolved as an increase in plasma glucose following capture. Plasma glucose levels were significantly higher 1 hour after capture (53.2 ± 4.6 mg 100 ml^{-1}) than at time 0 (37.2 ± 3.6 mg 100 ml^{-1}; $p<0.05$) and continued to rise during the subsequent three hours to peak at 89.8 ± 8.7 mg 100 ml^{-1} after four hours confinement. Glucose levels declined significantly between 4 and 5 hours to 72.6 ± 3.0 mg 100 ml^{-1}.

3.2.3 Plasma lactate

Plasma lactate levels for Experiment 4 are presented in Fig. 3.4 (c). Analysis of variance revealed a significant variation in the data with time. Lactate levels rose significantly ($p<0.001$) from time 0 (42.3 ± 4.2 mg 100 ml^{-1}) to reach a level of 88.7 ± 8.7 mg 100 ml^{-1} within one hour of capture. There was little subsequent change in lactate levels during the remaining four hours of confinement, levels remaining significantly higher than those at time 0 throughout ($p<0.01$, $p<0.001$).

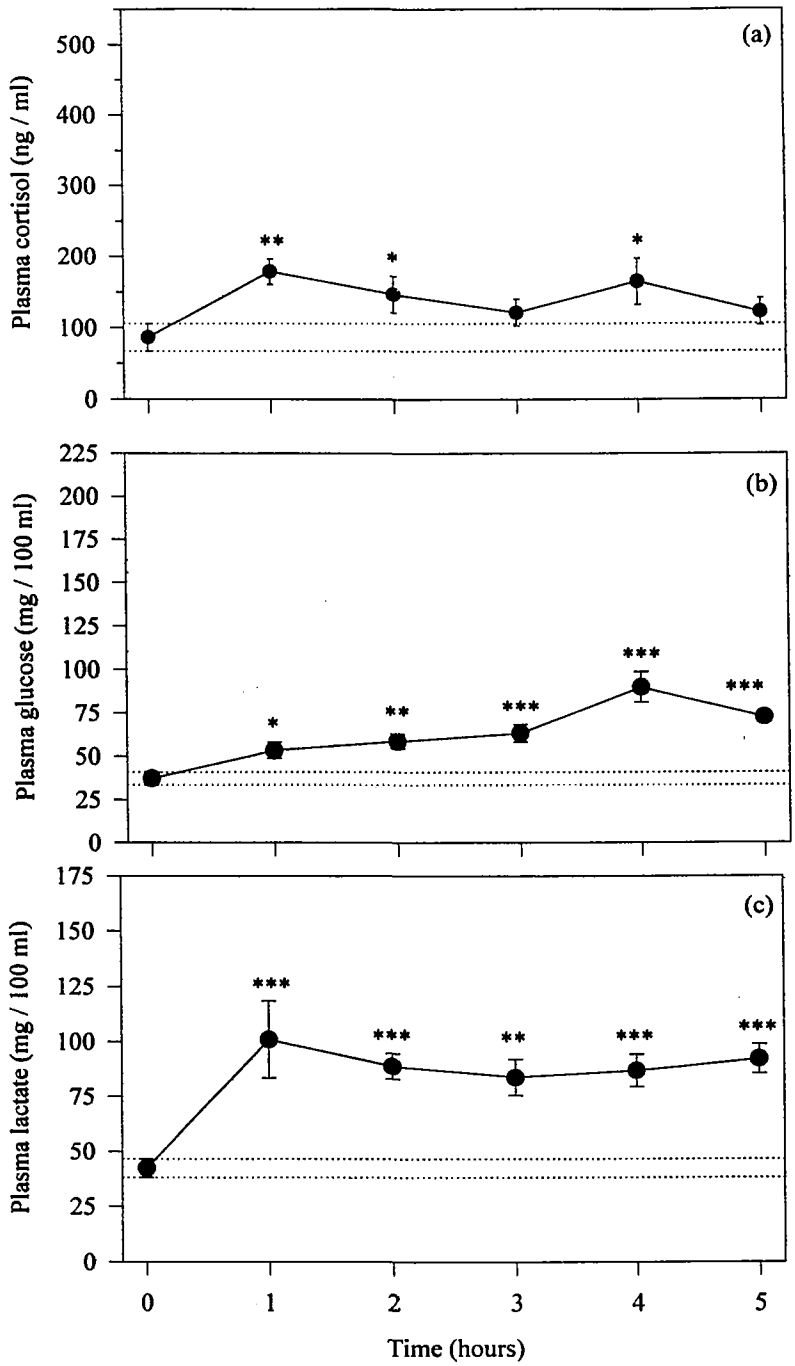

Figure 3. 4. Plasma cortisol, glucose, and lactate in rod-caught carp at intervals following capture and transfer to keepnets determined in Experiment 4. Plasma cortisol (a), plasma glucose (b) and plasma lactate (c) in rod-caught carp immediately following capture (0) and at five hourly intervals following unhooking and transfer to keepnets. Each point represents the mean ± SE, n = 10. Significant differences from values at time 0 are denoted by asterisks * $p<0.05$, ** $p<0.01$, *** $p<0.001$. The dotted line defines the mean at time 0 ± 1 standard error.

4. DISCUSSION

4.1 Effects of the Experimental Procedures on Plasma Cortisol Levels

The results clearly demonstrate that both capture and immediate release, and capture followed by a period of keepnet confinement, are stressful to carp. Plasma cortisol levels were significantly elevated within one hour of initial disturbance in all three laboratory-based experiments, indicating that the physical procedures to which the fish were subjected initiated the neuroendocrine "primary" stress response.

Two features of the response of the fish to these procedures are notable. In none of the experiments was there a statistically discernible difference in the response of the fish to capture alone when compared to capture followed by keepnet confinement. The magnitude and duration of cortisol elevation induced by both the experimental procedures was similar. Furthermore, the duration of the cortisol response was relatively short. Major perturbations in cortisol levels were observed only during the first four hours of the experiment. Although some minor fluctuations in cortisol levels occurred at 24 hours (Experiment 2), 48 hours (Experiment 1) and 168 hours (Experiment 3) after the start of the experiments, these were within the range of baseline levels and were also observed prior to the onset of disturbance (time 0, Experiments 1 and 3), suggesting they were unrelated to treatment.

The mean cortisol levels observed following stress in Experiments 1 to 3 (150-450 ng ml^{-1}), in which capture was simulated by netting and transfer to a second tank, are similar to, or higher than, those measured in rod-caught carp following capture and during keepnet confinement in Experiment 4 (75-200 ng ml^{-1}). This suggests that the experimental procedure constituted a stressor at least as severe as that of rod and line capture and was an appropriate approach to mimicking angling practice.

Mean plasma cortisol levels in Experiment 2 at one and four hours following the onset of stress (300-450 ng ml^{-1}) were markedly higher than those at the equivalent time points in Experiments 1 and 3 (50-300 ng ml^{-1}). The reasons for this are unclear. The difference may be related to the age or strain of the carp, rather than water temperature, because Experiments 1 and 3, between which there is little difference in absolute cortisol levels, were carried out at 4°C and 15°C respectively. Water temperature may, however, have influenced the dynamics of the cortisol response. In both Experiments 2 and 3, carried out at 15°C, high cortisol levels are achieved within one hour of the onset of disturbance and within a further three hours levels have begun to decline. In Experiment 1, which was carried out at a water temperature of 4°C, cortisol levels continue to rise until at least four hours after the onset of the stress. The fish employed in Experiment 2 were derived from a population which had experienced significant mortality due to an unidentified factor. However, negligible mortality occurred during the eight weeks prior to the experiment and the fish were feeding normally at the time of the experiment. Plasma cortisol levels at time 0 were comparable to those observed in the other experimental groups suggesting the fish were not experiencing additional stress. It is nonetheless possible that the previously observed mortality was selective for low-responding fish or that the agent responsible for the mortality had sensitised the remaining fish to stress.

Overall, the results suggest that the major determinant of the magnitude of the cortisol stress response in the experimental carp was the initial capture procedure. Only in Experiment 3 was there a significantly higher cortisol level in keepnet-confined fish compared to netted-only fish, at four hours after the start of the experiment (Fig. 3.3 (a)). At the other time-points and in the other experiments the cortisol response to capture followed by keepnet confinement was indistinguishable from the cortisol response to capture and release.

Most previous experimental work on the corticosteroid response of fish to stress has been carried out on salmonid fish, in particular the rainbow trout and Pacific salmon (Barton and Iwama 1991). There has been little work done on non-salmonid fish including cyprinids. However, what data are available suggest that the cortisol response to stress observed during the present study is characteristic of that observed in carp under a variety of stressful conditions. Both baseline pre-stress levels and levels of cortisol observed following capture in the present study are similar to those measured in carp before and following forced swimming (Leloup-Hatey 1960), handling (Ilan and Yaron 1976) and transport (Davis and Parker 1976). The magnitude and duration of the response to capture stress in the present study is also very similar to that observed in carp exposed to acid stress (van Dijk *et al.* 1993) in which cortisol levels rose from approximately 35 ng ml^{-1} prior to the onset of stress to peak at approximately 370 ng ml^{-1} within four hours before returning to baseline within 24 hours.

The absolute levels of cortisol observed in carp in the present study following handling are well in excess of those required to cause serious adverse effects on growth, reproduction, and immunity *if sustained over a prolonged period of time.* However, in the current study such levels were achieved only briefly (<24 hours) and there is no evidence in the literature to suggest that short exposure to elevated levels of cortisol constitute a threat to the well-being of the fish.

4.2 Effects of the Experimental Procedures on Plasma Glucose Levels

Plasma glucose levels are elevated during stress, largely, it is believed, as a consequence of the catecholaminergic response, although the involvement of cortisol has not been disproven. Plasma glucose levels are a function of many factors such as diet, age, time since feeding, and season, and therefore are a less unequivocal index of stress than cortisol (Wedemeyer *et al.* 1990). Although measurement of plasma glucose levels, a component of the secondary stress response, does not provide direct evidence of the adverse effects of stress in fish, quantification of plasma glucose does provide valuable complementary information regarding the severity and duration of the response, the time required for recovery from the stimulus, and provides another point of reference with previous work on the same and other species.

The effects of the experimental procedures on plasma glucose levels were less pronounced and more variable than the effects on plasma cortisol levels. Nonetheless, plasma glucose levels were significantly elevated by the experimental procedure in keepnet-confined fish in all four Experiments, and in netted fish during Experiment 3, providing further confirmation of the stressful nature of the experimental procedures. However, the changes in plasma glucose level were limited and occurred against a background of variation within and between samples not apparent with the plasma cortisol data.

The most marked effects arising from the experimental treatments were restricted to between 4 and 24 hours after the onset of the stress. No consistent evidence of significant treatment effects remained after 48 hours, although plasma glucose levels in keepnet-confined fish in Experiment 2 remained moderately elevated at 48 hours. Overall, capture and release, or capture and keepnet confinement, did not result in *major* perturbations in glucose levels, relative to the magnitude of change observed in plasma cortisol levels. This observation applies also to the data from rod-caught carp in which there was significant but moderate elevation in glucose levels during the period of confinement. The duration of the observed perturbations in plasma glucose following handling is consistent with the time-course of changes in plasma cortisol levels giving credence to the conclusion that the experimental manipulations elicited an acute stress response in the carp.

Glucose levels in unstressed fish in the present investigation (50 - 125 mg 100 ml^{-1}) fall within the range reported for carp in previous work (50 -100 mg 100 ml^{-1},Hertz *et al.* 1989, 45 - 65 mg 100 ml^{-1}, Blasco *et al.* 1992, 66 - 120 mg 100 ml $^{-1}$, Malinovskaya 1992) and the increase observed following stress in Experiments 2 and 3 is similar to that observed in carp subjected to handling associated with induction of ovulation (100 to 190 mg 100 ml^{-1}, Jeney *et al.* 1986). The time-course of changes in glucose level following capture is similar to that observed following exercise and handling stress in pike (Schwalme and Mackay 1985).

4.3 Effects of the Experimental Procedures on Plasma Lactate Levels

Elevated blood lactate levels arise through anaerobic respiratory activity in which glycogen stores are depleted and lactate accumulates in the muscle tissue (Milligan and Girard 1993). Thus lactate is not considered to be an indicator of stress *per se* but rather reflects the occurrence of exhaustive exercise. Lactate was measured during the present study to establish that the procedure employed to mimic capture and handling in Experiments 1 to 3 provided comparable physiological disturbance to that observed in rod-caught carp during Experiment 4, and to determine the time required for recovery from the respiratory effects of handling.

The levels of lactate measured in the present study (10 - 150 mg 100 ml^{-1}) are similar to those reported previously for other active fish species (see references in Milligan and McDonald 1988) and for carp; both baseline and post-stress lactate levels were within the range previously reported for carp before and following stress (33 - 51 mg 100 ml^{-1} prior to stress, 65 - 103 mg 100 ml^{-1} following stress; Dabrowska *et al.* 1991). While measurement of lactate alone does not provide an exact measure of the extent of metabolic acidosis, it is an adequate index of the duration of recovery (Pankhurst and Dedual 1994).

The most pronounced effects of the experimental procedures on plasma lactate levels were observed in Experiments 1 and 4, while only minimal treatment-related variation was apparent in lactate levels in Experiment 3, and none occurred in Experiment 2. In Experiment 1, lactate levels were elevated in both netted and keepnet-confined groups for a similar duration and to a similar extent, returning to baseline levels within 4-24 hours of the initial disturbance. In rod-caught carp, lactate levels rose within 1h of capture and remained significantly elevated for the duration of confinement. This suggests that, for Experiment 1 at least, the handling procedures employed produced metabolic disturbances similar to those occurring in rod-caught fish. Changes in lactate levels of similar magnitude and duration were observed in pike subjected to handling

and exercise (Schwalme and Mackay 1985).

Reasons for the inconsistency in response to handling are not immediately apparent. The physical procedures followed were identical for all three experiments. It is possible that the higher water temperature (15°C) prevailing during Experiments 2 and 3, compared to Experiment 1 (4°C) was a factor. The metabolic rate of fish, as poikilothermic organisms, is a direct function of their ambient temperature and lactate generated by anaerobic metabolism will be metabolised and cleared more rapidly at the higher temperature. However, it might equally be argued that higher water temperatures and greater metabolic activity might lead to more pronounced anaerobiosis. Unfortunately, there is no detailed information on lactate dynamics in carp with which to compare these data. Given these considerations, these results suggest that overall, handling with or without a subsequent period of keepnet confinement elicits similar perturbations in blood lactate levels and recovery is complete within 4 to 24h.

4.4 Other Research on Angling-Related Stress in Fish

There are few data with which to compare these results and none which specifically examine the effects of post-capture confinement. The physiological effects of capture by gill nets was examined in feral perch (Haux and Sjöbeck 1985). Both hyperglycaemia and hyperlactemia were observed as a consequence of capture, together with depletion of liver and muscle glycogen, lymphocytopenia and neutrophilia. Recovery, in terms of the return of these parameters to a stable baseline, required between two and four days. Obviously, although these data relate to the effects of removal of wild fish from their natural environment, capture by gill nets is not a procedure directly comparable to rod-and-line capture, and the fish were allowed to recover in tanks, not within their natural environment. Wydoski *et al.* (1976) examined the physiological response of rainbow trout to hooking and playing for up to 5 mins. The authors measured plasma osmolality, chloride levels, and plasma glucose. Consequent to hooking and playing, pronounced hyperglycaemia and hypochloremia were observed, and up to 72 hours were required for recovery. This period of recovery was not affected by temperature but hooking imposed greater stress on large fish than small fish. More recently, Pankhurst and Dedual (1994) studied the effects of capture by angling on rainbow trout and observed elevation of plasma lactate and cortisol following capture. They suggested that metabolic recovery occurred within 24 h of capture but that the post-capture holding conditions employed for experimental purposes were an additional source of stress which opposed recovery of cortisol levels to baseline. Finally, the implications of the brief exposure to air of exhaustively exercised rainbow trout for catch-and-release fisheries has been examined (Ferguson and Tufts 1992). These authors observed significant metabolic acidosis associated with capture and aerial exposure and concluded that the brief period of air exposure which occurs following capture is a significant additional stress above that of capture itself, and may have implications for survival of the fish.

None of these studies is directly comparable with the present work in terms of species or procedures, but nonetheless the results follow a similar pattern; capture of fish is stressful but recovery from this physiological disruption occurs within a period not exceeding 96h.

5. CONCLUSIONS

- Capture of carp from holding tanks by net, or from semi-natural conditions by rod and line, elicits a physiological stress response in the fish. This is characterised by a substantial elevation of plasma cortisol levels, a major index of the primary stress response, in captured carp compared to undisturbed carp.

- Transfer of carp to keepnets subsequent to capture does not increase or reduce the magnitude of the stress response observed.

- The duration of the cortisol response to capture, with or without subsequent confinement, is limited to between 4 and 24 hours after the onset of disturbance. This can be considered an acute, as opposed to a chronic, stress.

- The post-capture plasma cortisol elevation is accompanied by disturbances in plasma glucose and lactate levels but these are less consistent in severity and duration than the cortisol response. The changes in plasma glucose, as a component of the secondary stress response, provide confirmation that physiological disturbance following capture is limited and that recovery is rapid. Perturbations in blood lactate levels provide evidence of a limited degree of respiratory stress.

- In terms of physiological disruption, capture and confinement of fish for periods of up to four hours appears to constitute an acute, rather than chronic, stress. The stress response is essentially an adaptive mechanism and presents no threat to the well-being of the fish when short in duration. It is only under conditions of prolonged or chronic stress that adverse effects are observed.

- These data suggest that the retention of fish in keepnets following capture, under the experimental conditions employed, may not represent a source of stress additional to that imposed by capture and has no effect on the rate of recovery of the fish from the initial capture stress.

- This study was designed specifically to address the physiological aspects of capture and confinement and this conclusion does not therefore take account the possible effects of physical damage arising from the capture, playing, unhooking and confinement of rod-caught fish

6. RECOMMENDATIONS

The results reported here concerning the physiological stress associated with capture and keepnet confinement, and those of the previous study examining water quality changes within keepnets (Pottinger 1992), suggest that, *considering these factors only*, there is no firm basis for restricting the use of keepnets by anglers. However, several additional factors must be considered.

1. The present study is limited in terms of the experimental conditions employed and should be considered a first step in quantifying the physiological effects of angling practices on fish. Therefore, all the variables likely to influence the effects of capture and confinement were not examined.

   It is recommended that factors such as water temperature, water quality, and within-net stocking density, which are likely to influence the response of fish to the stress associated with capture and confinement, are addressed in future studies.

2. The present study is limited in terms of species. The reasons for employing common carp in the present study are listed in Section 1.3.3 and, as noted in Section 1.2.4, almost all the detailed work on the physiology of stress in fish has been carried out on salmonid fish. Although the salmonid appears to be a suitable model for most species so far studied, exceptions to this generalisation have been identified, such as tilapia (*Oreochromis mossambicus*; Balm *et al.*, 1994) and the sea raven (*Hemitripterus americanus*; Vijayan and Moon, 1994). There is, therefore, a requirement for reliable data on the nature of the stress response in other non-salmonid fish species, particularly species native to British waters.

   It is recommended that future work should characterise the response to, and effects of, stress in non-salmonid species of economic and recreational significance within the U.K.

3. The extent of physical damage incurred during capture, unhooking and keepnet confinement should be considered.

   It is recommended that future work should determine whether fish are physically damaged by such procedures, the extent of such damage, and the implications of angler-related damage for survival and well-being of the fish.

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