Journal of Fish Biology (2006) **69,** 613–621
doi:10.1111/j.1095-8649.2006.01109.x, available online at http://www.blackwell-synergy.com

Anaesthetization of Arctic charr *Salvelinus alpinus* (L.) with tricaine methanesulphonate or 2-phenoxyethanol for immediate blood sampling

J. S. Bystriansky*, P. J. LeBlanc† and J. S. Ballantyne*‡

*Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1 Canada and †Department of Phys. Ed. Kin., Brock University, St Catharines, Ontario, L2S 3A1 Canada

(Received 14 April 2005, Accepted 10 February 2006)

Anaesthetization of Arctic charr *Salvelinus alpinus* with tricaine methanesulphonate or 2-phenoxyethanol did not alter plasma protein, glucose, free amino acid, non-esterified fatty acid, ion or osmolality levels, suggesting that their use allows for the normal determination of these plasma variables.

Key words: amino acid; anaesthetic; glucose; non-esterified fatty acid; 2-phenoxyethanol; tricaine methanesulphonate.

The measurement of circulating blood metabolites is useful for monitoring the physiological and biochemical status of fishes. The stress associated with handling and blood sampling, however, can quickly alter the levels of some blood constituents. For example, stress has been shown to increase the levels of blood cortisol (Laidley & Leatherland, 1988), glucose (Vijayan & Moon, 1994), catecholamine and corticosteroid (Mazeaud *et al.*, 1977). Changes in the circulating levels of these metabolites can have significant effects on fish metabolism.

Anaesthetics have been employed to increase the ease of handling fishes and to reduce the effects of stress during blood sampling. Two anaesthetics used in fish research are tricaine methanesulphonate (TMS, or MS-222) and 2-phenoxyethanol (2-PE). Unfortunately, the use of these anaesthetics has themselves been shown to affect the levels of some blood variables. Both TMS and 2-PE have been found to increase cortisol levels in rainbow trout *Oncorhynchus mykiss* (Walbaum) (Barton & Peter, 1982), while TMS has been shown to alter plasma ion levels in plaice *Hippoglossoides platessoides* (Fabricius), dab *Pseudopleuronectes americanus* (Walbaum) and turbot *Reinhardtius hippoglossoides* (Walbaum) (Bourne, 1984); glucose levels in brook char *Salvelinus fontinalis* (Mitchill) (Houston *et al.*, 1971*a*), plaice and turbot (Bourne,

‡Author to whom correspondence should be addressed. Tel.: +1 519 824 4120 ex. 52708; fax: +1 519 767 1656; email: jballant@uoguelph.ca

1984) and plasma non-esterified fatty acid (NEFA) levels in rainbow trout (Harrington *et al.*, 1991), and induce haemo-concentration in carp *Cyprinus carpio* L. (Smit *et al.*, 1979*a*). To date, some reports on the effects of fish anaesthetics have been contradictory. Smit *et al.* (1979*a*) reported that TMS decreases plasma lactate levels in rainbow trout, while Black & Connor (1964) found no change. Also, the acidic properties of TMS when in solution cause changes in several blood variables not affected by buffered TMS (Allen & Harmon, 1970; Wedemeyer, 1970; Smit & Hattingh, 1979; Smit *et al.*, 1979*a*, *b*). Several studies have also reported interspecies differences in the effects of TMS on several blood variables (Smit & Hattingh, 1979; Smit *et al.*, 1979*a*, *b*; Bourne, 1984). Therefore, when choosing an anaesthetic, its effects on the blood variables being measured must be known for the species in question.

Arctic charr *Salvelinus alpinus* (L.) are an important aquaculture species in many areas of Europe and North America and are becoming a popular model species for fish research. The purposes of this study were first, to assess the effectiveness of TMS and 2-PE in reducing the stress related to blood sampling and second, to determine if the anaesthetics themselves affect the levels of several plasma metabolites in Arctic charr. To address these questions, several plasma variables were measured in Arctic charr anaesthetized with TMS or 2-PE and were compared to non-anaesthetized fish. Plasma glucose was determined, as its concentration is known to increase in response to stress (Barton & Iwama, 1991; Vijayan & Moon, 1994). Haemo-concentration is another common effect of stress (Barton & Iwama, 1991; Biron & Benfey, 1994) and was monitored by measuring plasma protein levels. Plasma ion and osmolality levels were measured to determine if the ionoregulatory ability of the Arctic charr was compromised by anaesthetization. Plasma NEFA and free amino acid (FAA) levels were monitored to assess any effect of anaesthetization on fish metabolism. Plasma NEFAs and FAAs are required for the synthesis of cell membranes and proteins, respectively, and both are important oxidative fuels in salmonids (Walton & Cowey, 1982). A change in their circulating concentration following anaesthetization could indicate a deviation from normal metabolism.

Arctic charr, mean $\pm$ S.E. mass of 672 $\pm$ 50 g, were kept in fresh water at 10° C in a 2000 l flow-through tank on a 12L:12D photoperiod. Fish were fed with trout chow pellets (Martin Feeds, Elmira, Ontario, Canada) up to 24 h before the start of the experiment. Fish were randomly selected from a stock tank and placed in a 60 l covered anaesthetic bath that contained one of two treatments; TMS (100 $mg \cdot l^{-1}$ TMS buffered with 100 $mg \cdot l^{-1}$ $NaHCO_3$) or 2-PE (400 $\mu l \cdot l^{-1}$). Buffering TMS with bicarbonate was necessary to maintain neutral water pH (7·3). Fish remained in the anaesthetic bath until they lost equilibrium, which was *c.* 2 min for anaesthetization with TMS and 3 min for anaesthetization with 2-PE. Care was taken to maintain all fish as unstressed as possible. The anaesthetic bath was large enough for the Arctic charr to swim around, covered to reduce light and visual stimuli and maintained at a constant temperature of 10° C. Immediately following loss of equilibrium, fish were removed and blood was sampled by caudal puncture using a heparinized (500 U ml^{-1} sodium heparin) syringe and 21 G needle. Arctic charr representing a control group were quickly netted and sampled without anaesthetization.

This unanaesthetized control group was considered unstressed and used to assess resting values for the plasma metabolites determined. Control fish were also maintained as unstressed as possible and sampling time was <20 s for all individuals. Blood was centrifuged at 3000 *g* for 10 min at 4° C. Plasma was removed, frozen in liquid nitrogen and stored at −80° C until analysed.

Specific methylation, to fatty acid methyl esters, and determination of plasma NEFAs, using a gas chromatograph (Hewlett-Packard, HP5890A) fitted with a flame ionization detector, an automatic injector (Hewlett-Packard, 7673A) and a DB-225 megabore fused silica column (Chromatographic Specialities Inc., Brockville, Ontario, Canada), were performed as previously described by Ballantyne *et al.* (1993). Fatty acid methyl esters from plasma samples were identified by comparing their retention times with known standards, and the absolute amounts were quantified with the aid of an internal standard, heptadecanoic acid (17:0), added to the plasma samples prior to methylation.

Determination of plasma FAAs, using a high-performance liquid chromatography (HPLC) (Hewlett-Packard, HP 1090 series II/L liquid chromatograph) equipped with a ultraviolet visible series II diode array detector, an automatic injector and a narrow bore (200 × 2·1 mm) reversed phase column (Amino-Quant 79916AA-572; Hewlett-Packard), was carried out as outlined by Barton *et al.* (1995). Amino acids from plasma samples were identified by comparing their retention times with known standards, and the absolute amounts were quantified with the aid of internal standards, norvaline and azetidine-2-carboxylic acid, added to the plasma samples prior to analysis.

Osmolality was determined using a vapour pressure osmometer (Model 5500; Wescor, Logan, UT, U.S.A.). Chloride levels were measured using a chloride titrator (Model CMT10; Radiometer, Copenhagen, Denmark). Sodium and potassium levels were measured using a flame photometer (Model FLM2; Radiometer). Glucose was determined using a Sigma diagnostic kit (Sigma, St Louis, MO, U.S.A.). Plasma protein was measured using a Bio-Rad protein assay (Bio-Rad, Hercules, CA, U.S.A.) standardized with bovine serum albumin (BSA). All chemicals used were purchased from Sigma Chemical Co. (Sigma-Aldrich, Canada Ltd, Oakville, Ontario, Canada), with the exception of the BSA (purchased from BioShop, Burlington, Ontario, Canada), HPLC grade methanol and acetonitrile (purchased from Fisher Scientific Ltd, Whitby, Ontario, Canada) and fatty acid standards (purchased from Nu Check Prep Inc., Elysian, MN, U.S.A.). All chemicals used were of the highest available purity and purchased from Sigma Chemical Co. (Sigma-Aldrich, Canada Ltd), with the exception of the TMS (purchased from ICN pharmaceuticals, Cleveland, OH, U.S.A), BSA (purchased from BioShop), methanol and chloroform (purchased from Fisher Scientific Ltd) and fatty acid standards (purchased from Nu Check Prep Inc.). An ANOVA (Steel & Torrie, 1980) was used to establish differences between the control and the two treatment groups for total and individual plasma NEFAs and FAA, osmolality, protein, ions and glucose. LSMEANS (SAS Institute Inc., Cary, NC, U.S.A) was used to determine significance ($P < 0{\cdot}05$). Assumptions for normality, independence and homeoscedasticity were verified by generating appropriate residual plots. Data transformations ($\log_{10}$, square root and inverse square root) were used when appropriate to meet the above assumptions.

Anaesthesia with either TMS or 2-PE (at the employed dose and duration) showed no effect on levels of blood metabolites of the two treatment groups compared to those of the control fish or between the treatments. Plasma NEFAs (Table I) and FAAs (Table II) were not significantly different between the control and the two treatment groups or between the treatment groups. Anaesthetization with TMS or 2-PE did not affect plasma ion (Na^+, Cl^- and K^+), osmolality, protein or glucose levels, as they were not significantly different from that of control fish or between the two treatments groups (Table III).

The results of this study indicate that anaesthetization of Arctic charr with TMS or 2-PE does not alter circulating plasma osmolality, ion, protein or glucose levels and does not significantly change the plasma FAA or NEFA content if blood samples are collected within 2–3 min after initial exposure. An increase in plasma glucose level is a common secondary response to stress in fishes (Barton & Iwama, 1991). Control Arctic charr exhibited plasma glucose levels that were similar to resting levels found in other salmonids (Hille, 1982). This suggests that the fish in the control group were unstressed. Anaesthesia with TMS has been shown to increase plasma glucose levels in several fish species (Houston *et al.*, 1971*a*, *b*; Smit *et al.*, 1979*a*; Bourne, 1984) within several minutes. Pucéat *et al.* (1989) found that hepatocytes isolated from rainbow trout anaesthetized with TMS produced and released significantly more glucose than from those anaesthetized with 2-PE. This was attributed to higher rates of glycogenolysis due to increased stress levels in TMS-anaesthetized rainbow trout. Plasma glucose levels of Arctic charr anaesthetized with TMS or 2-PE were not different from those of control fish, suggesting that under these conditions, anaesthetization did not elevate stress levels and had no direct effect on plasma glucose levels. Morales *et al.* (1990) found that handling stress induced an increase in rainbow trout plasma glucose and FAA levels, but anaesthetization with TMS prevented this effect. Morales *et al.* (1990) suggested that the elevation in plasma glucose was supported by the metabolism of amino acids as gluconeogenic precursors. As plasma glucose levels in this study were stable following anaesthetization, no change in the use of FAAs for gluconeogenesis would be expected. This study found that neither TMS nor 2-PE altered plasma FAA levels in Arctic charr, suggesting that overall rates of FAA oxidation, protein synthesis and protein degradation were unchanged during anaesthesia. The concentrations of individual and total plasma FAAs found in control Arctic charr in this study were similar to those in other studies on Arctic charr (Barton *et al.*, 1995; Bystriansky, 2005).

Plasma NEFAs are the most metabolically active form of lipid in the blood of vertebrates and their levels indicate the degree to which fatty acids are used as a fuel source (Henderson & Tocher, 1987). The NEFAs can also be incorporated into cellular membranes and are important precursors to many hormones and eicosanoids. The levels of individual and total plasma NEFAs were not different in anaesthetized Arctic charr compared to those in control fish, suggesting that neither TMS nor 2-PE had a direct effect on their metabolism. Mazeaud *et al.* (1977) found that both hypoxia and an injection of adrenaline caused a rapid decline in plasma free fatty acids and suggested that this decrease was a secondary effect of stress. Interestingly, stress is also known to cause an increased mobilization of lipid from the liver of fishes (Jezierska

TABLE I. Absolute amounts (mean ± S.E., $n = 8$) of individual non-esterified fatty acids in the plasma of non-anaesthetized (control) and anaesthetized *Salvelinus alpinus*

	Concentration ($nmol \cdot ml^{-1}$)		
Fatty acid	Control	Tricaine methanesulphonate	2-phenoxyethanol
Saturates			
14:0	59·9 ± 12·2	60·4 ± 11·6	41·2 ± 8·8
16:0	528·3 ± 56·1	534·0 ± 61·7	426·8 ± 63·2
18:0	91·4 ± 15·4	88·4 ± 12·5	69·5 ± 15·0
20:0	3·3 ± 1·0	2·7 ± 0·7	6·2 ± 3·1
22:0	2·0 ± 1·8	bld	2·6 ± 1·7
23:0	7·2 ± 1·4	7·7 ± 1·4	5·2 ± 1·1
24:0	bld	bld	0·6 ± 0·6
Monoenes			
14:1	4·1 ± 1·1	4·3 ± 1·5	1·4 ± 0·3
16:1	164·5 ± 28·9	170·7 ± 28·1	130·9 ± 25·4
18:1	430·6 ± 58·8	480·8 ± 75·3	393·5 ± 70·9
20:1	60·2 ± 10·7	67·3 ± 18·5	69·2 ± 13·4
22:1	22·7 ± 6·5	28·1 ± 7·8	26·2 ± 4·7
24:1	7·1 ± 2·0	5·6 ± 2·3	2·6 ± 1·4
Polyenes			
18:2n6	81·9 ± 9·3	111·6 ± 25·9	92·6 ± 15·4
18:3n3	17·5 ± 1·8	21·7 ± 3·6	17·8 ± 1·9
18:4n3	32·0 ± 3·8	37·6 ± 6·1	29·5 ± 5·0
20:2n6	10·8 ± 3·7	8·6 ± 2·5	12·0 ± 3·6
20:3n6	4·2 ± 1·1	5·0 ± 1·2	4·9 ± 1·3
20:4n6	30·3 ± 4·9	32·5 ± 5·3	23·6 ± 5·6
20:3n3	1·2 ± 0·8	0·5 ± 0·3	1·0 ± 0·8
20:4n3	9·3 ± 1·7	9·3 ± 2·4	4·9 ± 1·8
20:5n3	275·2 ± 44·8	342·2 ± 60·0	188·1 ± 34·5
22:2n6	0·3 ± 0·2	bld	bld
22:4n6	1·6 ± 1·0	0·4 ± 0·3	1·4 ± 1·4
22:5n6	20·8 ± 4·8	30·4 ± 8·9	16·0 ± 5·8
22:5n3	66·0 ± 14·7	68·4 ± 15·9	55·2 ± 14·5
22:6n3	321·0 ± 39·5	330·1 ± 47·8	252·8 ± 47·6
Total	2253·3 ± 279·9	2448·1 ± 326·2	1875·55 ± 301·9
Total saturates	692·1 ± 84·0	693·2 ± 86·2	552·0 ± 87·3
Total monoenes	689·3 ± 90·1	756·8 ± 118·3	623·7 ± 106·5
Total polyenes	871·9 ± 112·1	998·1 ± 149·8	699·8 ± 119·1
n3	722·2 ± 100·1	809·7 ± 130·7	549·3 ± 101·5
n6	149·7 ± 14·4	188·4 ± 30·1	150·5 ± 22·8

bld, below level of detection.

et al., 1982), which should result in a rise in circulating NEFA levels. Harrington *et al.* (1991) showed that anaesthetization with TMS induced a 30% decrease in total plasma NEFAs levels from rainbow trout within 30 s. These changes were attributed to an antilipolytic effect of TMS, which would decrease the rate of NEFAs released from the liver into the circulation. The decrease in

TABLE II. Absolute amounts (mean ± S.E., $n = 8$) of individual amino acids in the plasma of non-anaesthetized (control) and anaesthetized *Salvelinus alpinus*

	Concentration ($nmol \cdot ml^{-1}$)		
Amino acid	Control	Tricaine methanesulphonate	2-phenoxyethanol
Essential			
Histidine	179·6 ± 25·2	133·8 ± 23·1	158·7 ± 15·8
Threonine	338·6 ± 24·4	259·2 ± 23·6	318·2 ± 23·3
Valine	525·4 ± 30·6	491·6 ± 55·2	514·5 ± 27·3
Methionine	187·9 ± 24·4	190·6 ± 32·1	150·7 ± 13·2
Tryptophan	89·1 ± 7·4	95·8 ± 11·7	89·7 ± 6·2
Phenylalanine	120·4 ± 8·3	121·9 ± 18·9	99·7 ± 4·7
Isoleucine	215·1 ± 16·6	194·0 ± 22·3	201·6 ± 16·1
Leucine	388·2 ± 24·0	350·4 ± 43·1	346·5 ± 21·9
Lysine	319·6 ± 42·3	322·3 ± 64·4	331·7 ± 50·5
Non-essential			
Aspartate	199·4 ± 22·5	163·8 ± 21·9	173·9 ± 8·3
Glutamate	81·6 ± 18·5	73·1 ± 11·1	77·0 ± 12·2
Asparagine	135·4 ± 18·2	99·5 ± 17·5	94·1 ± 6·9
Serine	92·4 ± 9·5	73·8 ± 11·2	76·5 ± 8·1
Glutamine	325·2 ± 16·7	255·4 ± 35·3	287·0 ± 14·7
Glycine	264·8 ± 40·6	189·7 ± 30·4	221·1 ± 25·8
Alanine	333·8 ± 36·2	327·7 ± 37·9	385·5 ± 50·2
Taurine	300·3 ± 36·7	456·8 ± 74·1	502·0 ± 56·4
Tyrosine	140·9 ± 26·3	88·2 ± 12·0	95·0 ± 11·2
Arginine	219·2 ± 27·5	159·0 ± 17·5	170·8 ± 19·8
Hydroxyproline	172·0 ± 29·9	179·1 ± 40·9	128·3 ± 30·2
Proline	138·6 ± 25·7	127·5 ± 20·9	132·4 ± 13·3
Total amino acids	4767·5 ± 230·0	4353·4 ± 490·0	4554·8 ± 176·3
Essential amino acids	2363·9 ± 121·5	2159·7 ± 232·7	2211·3 ± 118·2
Non-essential amino acids	2403·6 ± 152·8	2193·7 ± 262·4	2243·5 ± 80·7

plasma NEFAs found by Harrington *et al.* (1991) may also be due to the use of non-buffered TMS in that study. Buffering the water is necessary as non-buffered TMS causes a significant decrease in water pH (Allen & Harmon, 1970), which has been shown to increase stress levels and have detrimental effects on fishes (Wedemeyer, 1970; Smit & Hattingh, 1979; Smit *et al.*, 1979*a*, *b*).

Several studies have reported that handling stress (Biron & Benfey, 1994) or anaesthetization with TMS (Houston *et al.*, 1971*a*; Reinitz & Rix, 1977; Smit *et al.*, 1979*a*; Lowe-Jinde & Niimi, 1983; Laidley & Leatherland, 1988) induces haemo-concentration in fishes. In this study, plasma protein levels were used to indicate changes in plasma concentration. Levels of plasma protein in control Arctic charr were similar to resting levels found in several salmonid species (Hille, 1982). Anaesthetization with TMS or 2-PE induced no change in plasma protein levels in Arctic charr, suggesting that anaesthesia did not alter the overall concentration of the plasma. The haemo-concentrating effects of stress and

TABLE III. Osmolality, ion and glucose levels (means ± S.E., $n = 8$) in the plasma of non-anaesthetized (control) and anaesthetized *Salvelinus alpinus*

	Control	Tricaine methanesulphonate	2-phenoxyethanol
Protein ($mg \cdot ml^{-1}$)	41·1 ± 2·4	40·6 ± 2·1	40·5 ± 2·4
Osmolality ($mOsm \cdot kg^{-1}$)	307·1 ± 6·7	298·0 ± 2·0	305·4 ± 5·8
Chloride ($mEq \cdot l^{-1}$)	115·8 ± 2·9	116·0 ± 3·6	117·3 ± 4·0
Sodium ($mEq \cdot l^{-1}$)	143·5 ± 3·3	140·8 ± 1·6	143·1 ± 4·6
Potassium ($mEq \cdot l^{-1}$)	2·9 ± 0·1	2·7 ± 0·1	2·9 ± 0·1
Glucose ($mmol \cdot l^{-1}$)	4·95 ± 0·27	4·74 ± 0·27	4·14 ± 0·21

anaesthetics are often accompanied by increases in plasma ion and osmolality levels (Houston *et al.*, 1971*a*; Smit *et al.*, 1979*a*). Plasma osmolality, sodium, potassium and chloride levels were not different between anaesthetized and control fish and were very similar to those found in other studies on freshwater Arctic charr (Bystriansky, 2005), indicating that their ionoregulatory status was not compromised by anaesthetization.

The results of this study confirm that anaesthetization with both TMS and 2-PE at the dose and duration employed here allow for the accurate determination of resting levels of plasma glucose, FAAs, NEFAs and ions if blood samples are obtained immediately following loss of equilibrium. Caution should be taken, as other studies have reported that many other factors may affect anaesthetization. Anaesthetic dose (Smit & Hattingh, 1979), water hardness (Dawson & Marking, 1973; Gilderhus *et al.*, 1973), temperature (Weyl *et al.*, 1996) and fish size (Oikawa *et al.*, 1994) have all been shown to affect times to anaesthetization and may induce different physiological reactions. Smit *et al.* (1979*a*) even showed that fishes of similar size of the same species do not respond identically to TMS, with times to anaesthetization varying between individuals. The specific effects of anaesthesia should be known for the species in question. This study does not consider the longer term effects of the TMS or 2-PE on fish metabolism as changes to the plasma metabolites measured in this study may occur at some point following anaesthesia. It is recommended that the effects of repeated anaesthesia, longer durations of anaesthesia or use of stronger anaesthetic dose be determined for the biochemical variable being determined.

This research was funded by a NSERC Strategic grant and NSERC Discovery grant to J. S. Ballantyne. Arctic charr were generously donated by the Alma Aquaculture Research station (Alma, Ontario, Canada).

References

Allen, J. L. & Harmon, P. D. (1970). Control of pH in MS 222 anaesthetic solutions. *Progressive Fish-Culturist* **32,** 100.

Ballantyne, J. S., Glemet, H. C., Chamberlin, M. E. & Singer, T. D. (1993). Plasma nonesterified fatty acids of marine teleost and elasmobranch fishes. *Marine Biology* **116,** 47–52.

Barton, B. A. & Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* **1,** 3–26.
Barton, B. A. & Peter, R. E. (1982). Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia, and cold shock. *Journal of Fish Biology* **20,** 39–51.
Barton, K. N., Gerrits, M. F. & Ballantyne, J. S. (1995). Effects of exercise on plasma nonesterified fatty acids and free amino acids in Arctic char (*Salvelinus alpinus*). *Journal of Experimental Zoology* **271,** 183–189.
Biron, M. & Benfey, T. J. (1994). Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout (*Salvelinus fontinalis* Mitchell). *Fish Physiology and Biochemistry* **13,** 153–160.
Black, E. C. & Connor, A. R. (1964). Effects of MS 222 on glycogen and lactate levels in rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **21,** 1539–1542.
Bourne, P. K. (1984). The use of MS 222 (tricaine methanesulphonate) as an anaesthetic for routine blood sampling in three species of marine teleosts. *Aquaculture* **36,** 313–321.
Bystriansky, J. S. (2005). Regulation of gill Na^+,K^+-ATPase during salinity acclimation of salmonid fishes. PhD Thesis, University of Guelph, Guelph, Ontario.
Dawson, V. K. & Marking, L. L. (1973). Toxicity of mixtures of quinaldine sulfate and MS-222 to fish. *U.S. Department of Fish and Wildlife Service* **53,** 3–11.
Gilderhus, P. A., Berger, B. L., Sills, J. B. & Harman, P. D. (1973). The efficacy of quinaldine sulfate:MS-222 mixtures for the anesthetization of freshwater fish. *U.S. Department of the Interior Publication* **54,** 1–9.
Harrington, A. J., Russell, K. A., Singer, T. D. & Ballantyne, J. S. (1991). The effects of tricaine methanesulphonate (MS-222) on plasma nonesterified fatty acids in rainbow trout, *Oncorhynchus mykiss*. *Lipids* **26,** 774–775.
Henderson, R. J. & Tocher, D. R. (1987). The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* **26,** 281–347.
Hille, S. (1982). A literature review of the blood chemistry of rainbow tout, *Salmo gairdneri* Rich. *Journal of Fish Biology* **20,** 535–569.
Houston, A. H., Madden, J. A., Woods, R. J. & Miles, H. M. (1971*a*). Some physiological effects of handling and tricaine methanesulphonate anesthetization upon the brook trout, *Salvelinus fontinalis*. *Journal of the Fisheries Research Board of Canada* **28,** 625–633.
Houston, A. H., Madden, J. A., Woods, R. J. & Miles, H. M. (1971*b*). Variations in blood and tissue chemistry of brook trout, *Salvelinus fontinalis*, subsequent to handling, anesthesia, and surgery. *Journal of the Fisheries Research Board of Canada* **28,** 635–642.
Jezierska, B., Hazel, J. R. & Gerking, S. D. (1982). Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acids. *Journal of Fish Biology* **21,** 681–692.
Laidley, C. W. & Leatherland, J. F. (1988). Cohort sampling, anaesthesia and stocking-density effects on plasma cortisol, thyroid hormone, metabolite and ion levels in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **33,** 73–81.
Lowe-Jinde, L. & Niimi, A. J. (1983). Influence of sampling on the interpretation of haematological measurements of rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* **61,** 396–402.
Mazeaud, M. M., Mazeaud, F. & Donaldson, E. M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society* **106,** 201–212.
Morales, A. E., Garcia-Rejón, L. & De La Higuera, M. (1990). Influence of handling and/or anaesthesia on stress response in rainbow trout. Effects on liver primary metabolism. *Comparative Biochemistry and Physiology* **95A,** 87–93.
Oikawa, S., Takeda, T. & Itazawa, Y. (1994). Scale effects of MS-222 on a marine teleost, porgy *Pagrus major*. *Aquaculture* **121,** 369–379.

Pucéat, M., Garin, D. & Fréminet, A. (1989). Inhibitory effect of anaesthesia with 2-phenoxyethanol as compared to MS222 on glucose release in isolated hepatocytes from rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology* **94A,** 221–224.

Reinitz, G. L. & Rix, J. (1977). Effect of tricaine methanesulphonate (MS222) on hematocrit values in rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology* **56C,** 115–116.

Smit, G. L. & Hattingh, J. (1979). Anaesthetic potency of MS 222 and neutralized MS 222 as studied in three freshwater fish species. *Comparative Biochemistry and Physiology* **62C,** 237–241.

Smit, G. L., Hattingh, J. & Burger, A. P. (1979*a*). Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species: interspecies differences. *Journal of Fish Biology* **15,** 633–643.

Smit, G. L., Hattingh, J. & Burger, A. P. (1979*b*). Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species: haemoglobin electrophoresis, ATP levels and corpuscular fragility curves. *Journal of Fish Biology* **15,** 655–663.

Steel, R. G. D. & Torrie, J. H. (1980). *Principles and Procedures of Statistics: a Biometrical Approach*. New York: McGraw-Hill Book Company.

Vijayan, M. M. & Moon, T. W. (1994). The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Canadian Journal of Zoology* **72,** 379–386.

Walton, M. J. & Cowey, C. B. (1982). Aspects of intermediary metabolism in salmonid fish. *Comparative Biochemistry and Physiology* **73B,** 59–79.

Wedemeyer, G. (1970). Stress of anesthesia with M.S. 222 and benzocaine in rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **27,** 909–914.

Weyl, O., Kaiser, H. & Hecht, T. (1996). On the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for goldfish, *Carassius auratus* (L.), at different temperatures and concentrations. *Aquaculture Research* **27,** 757–764.