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Statistical Relations of Some Blood Parameters Along Recovery from Imposed Stress in Dogfish

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Selected blood parameters (arterial pH, O_2 and CO_2 tensions, oxygen content, bicarbonate and lactate concentrations, haematocrit, haemoglobin, red blood cell count, mean corpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration), oxygen consumption and ventilatory frequency were measured 0, 3 and 24 hours after cannulation of the dorsal aorta of 22 dogfish. Correlations were calculated between all pairs of variables along the post-operational recovery period. Results are discussed in terms of the integrated dynamics of the recovery process subsequent to handling, anaesthesia and surgery stress. It is suggested that erythrocyte swelling plays an important role in maintaining tissue oxygen supply during recovery from operational procedures.

Key words: Blood parameters, Imposed stress.

There is an increasing awareness that fish can display a wide range of behavioural, systemic and cellular responses to a variety of sublethal stressors (7). A complicatory source of stress in fish physiology studies arises from the operational and experimental procedures employed such as handling, anaesthesia, insertion of electrodes and cannulae. Consequently, a knowledge of the effects of such procedures and the time course required to reattain normal levels is essential in order to permit the valid interpretation of results obtained from further experimental challenges.

Studies of the effects of anaesthesia and cannulation on the haematological and respiratory status of teleosts have been performed (13, 14). The effects of operational stress on the elasmobranch *Scyliorhinus canicula* over a 24 h recovery period were recently assessed (4). Using the standard statistical procedure of Student t-test, it was concluded that, following an initial internal hypoxia, the majority of respiratory and haematological variables attained stable levels within 3 h post-operation, exceptions being blood pH and lactate.

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There has been recent criticism of the use of t-test in studies where the experimental design involves multiple comparisons (5). Consequently, despite the very common use of t-test in the context of assessing significant changes over time, we have completed this analysis using the technique of Pearson correlation coefficients. Correlations have been calculated between selected parameters at different sampling times following insertion of dorsal aortic cannulae in order to assess whether such a statistical procedure will fundamentally alter the conclusions previously found (4). In addition, such an analysis by permitting an integrative approach may elucidate some previously undisclosed observations on the dynamics of the haematological and respiratory adjustments along the recovery period subsequent to cannulation.

Materials and Methods

A detailed description of the experimental techniques employed can be found in a previous work (4). In brief, dogfish (mean weight ± S.D.: 214 ± 41 g) were caught in the Mediterranean near Barcelona and maintained in aquaria (Acuario, Instituto de Investigaciones Pesqueras de Barcelona), for six weeks prior to experimentation. Fish were anaesthetised in MS-222 anaesthetic (0.1 g l^{-1}) and cannulated in the dorsal aorta. Blood samples (0.5 ml) were removed immediately following the operation and 3 and 24 h later, the fish being placed in flow-through respirometers during the recovery period.

pHa,* PaO_2, CaO_2 and $CaCO_2$ were measured by electrode techniques. pHa was determined using a BMS 3 Mk 2 blood microsystem, calibrated with Radiometer buffers. PaO_2 was obtained with a Radiometer E5047 electrode thermostatted in a D616 cuvette. CaO_2 and $CaCO_2$ were measured according to TUCKER (15) and CAMERON (2) methods respectively. Bicarbonate concentration was derived from the Henderson-Hasselbach equation. Lactate and haemoglobin (Hb) concentrations were found by means of adequate commercial kits (Boehringer-Mannheim). Vf was determined visually and VO_2 was calculated from oxygen tensions of inlet and outlet water, flow rate and the solubility of O_2 at the experiment temperature.

Statistical analysis were performed with a VAX/Digital Computer using an SPSS-X program for Pearson correlation coefficients. Three types of statistical category were assumed: highly significant ($p < 0.001$), significant ($p < 0.05$) and indicative ($p < 0.1$). A positive correlation indicates a related increase of one parameter with another. A negative correlation implies an increase in one parameter corresponds with a significant decrease in the other.

Results

The values for selected haematological and respiratory parameters from the dogfish were determined immediately after the operational procedures and after 3 and 24 hours (table I). Unlike other works (4), standard deviations are employed in preference to standard errors as the former gives a more accurate summarisation of the variability in the observations (5). It can be concluded that immediately after operational procedures pHa, PaO_2 and CaO_2 show reduced values and $CaCO_2$, bicarbonate and plasma lactate increased. Erythrocyte swelling is apparent. However, with the exception of pHa and lactate, most measured variables attained a stable level within 3 h.

In the present study, as an alternative to Student t-test, two types of correla-

* Abbreviations (see table I).

Table I. *Measured variables at the three sampling times: Mean, standard deviation (SD), standard error (SEM) and sample size (N).*

	0 h sample				3 h sample				24 h samble			
	Mean	SD	SEM	N	Mean	SD	SEM	N	Mean	SD	SEM	N
VO_2 (ml O_2 kg^{-1} h^{-1})	31	12.6	4	10	24	5.6	2	8	20	7.9	3	7
Vf (beats min^{-1})	72	10.4	3	12	73	10.4	3	12	67	10.6	4	7
PaO_2 (mm Hg)	43.6	36.8	11.1	11	94.8	23.1	7.7	9	92.9	17.4	5.8	9
CaO_2 (vol %)	2.0	1.3	0.4	11	3.6	1.2	0.4	9	3.1	0.9	0.3	9
$CaCO_2$ ($mmoll^{-1}$)	9.8	4.8	1.8	7	4.0	0.9	0.4	6	3.2	1.1	0.5	5
HCO_3^- ($mEql^{-1}$)	7.6	3.7	1.4	7	3.4	0.7	0.3	6	2.7	1.1	0.5	5
pHa	7.193	0.13	0.04	10	7.479	0.1	0.036	8	7.59	0.08	0.028	8
Lactate ($mmoll^{-1}$)	9.0	4.7	1.5	10	9.1	5.0	1.9	7	5.6	2.1	0.8	7
MCV (μm^3)	1758	430	136	10	1313	360	136	7	1240	376	133	8
MCH (pg)	1264	654	207	10	783	252	95	7	750	254	90	8
MCHC (%)	76.7	13.6	4.3	10	59.9	21.8	7.7	8	61.6	16.1	5.7	8
Hb (g100 ml^{-1})	13.1	3.0	0.9	11	8.2	4.5	1.6	8	7.3	2.3	0.8	8
RBCC (millionmm^{-3})	0.107	0.03	0.008	10	0.116	0.05	0.018	7	0.103	0.03	0.011	8
Ht (%)	17	2.4	0.7	12	13	3.0	1.0	9	12	2.7	0.9	9

Abbreviations: $CaCO_2$ = Arterial CO_2 Content; CaO_2 = Arterial O_2 Content; Hb = Haemoglobin; Ht = Haematocrit; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; MCV = Mean Corpuscular Volume; PaO_2 = Arterial Oxygen Pressure (tension); pHa = Arterial pH; RBCC = Red Blood Cell Count; Vf = Ventilatory Frequency; VO_2 = Oxygen Consumption.

Table II. *Highly significant, significant and indicative Pearson correlation coefficients for 0, 3 and 24 hour parameter pairs.*
Abbreviations as in table I.

0 h sample		3 h sample		24 h sample	
		Highly significant correlations			
$CaCO_2$ — HCO_3^-	0.9984	$CaCO_2$ — HCO_3^-	0.9988	$CaCO_2$ — HCO_3^-	0.9994
		Hb — MCHC	0.9725		
		Significant correlations			
Ht — pHa	—0.7220	$CaCO_2$ — pHa	—0.7713	PaO_2 — Ht	—0.8130
RBCC — PaO_2	—0.6338	MCH — HCO_3^-	0.8099	MCV — lactate	0.7972
Vf — lactate	0.5635	RBCC — Ht	0.7370	RBCC — MCV	—0.6486
Hb — MCHC	0.7303	RBCC — Hb	0.7088	MCV — MCH	0.6904
Hb — Vf	0.6121	MCH — $CaCO_2$	—0.7914	VO_2 — MCHC	—0.7545
RBCC — MCV	—0.7763	Ht — Hb	0.8518	VO_2 — Vf	—0.8125
CaO_2 — PaO_2	0.7568	Ht — MCHC	0.7268	CaO_2 — lactate	0.8169
Hb — lactate	0.6346	RBCC — MCV	—0.9052	MCH — lactate	0.7115
Ht — Hb	0.6528			RBCC — Vf	0.7553
Hb — MCH	0.7258			MCV — Vf	—0.8169
				VO_2 — MCH	—0.7788
		Indicative correlations			
Hb — pHa	—0.5333	pHa — HCO_3^-	—0.7470	PaO_2 — Hb	—0.5724
CaO_2 — $CaCO_2$	0.5819	$CaCO_2$ — CaO_2	—0.6477	PaO_2 — RBCC	—0.5934
Vf — CaO_2	—0.5256	RBCC — CaO_2	0.5706	$CaCO_2$ — lactate	0.8600
MCH — MCHC	0.5195	Hb — MCH	0.5845	lactate — Vf	—0.5783
MCH — lactate	0.5141	MCH — MCHC	0.6282	Ht — RBCC	0.5789
MCV — PaO_2	0.5452	pHa — lactate	—0.5956	Hb — MCH	0.5374
HCO_3^- — CaO_2	0.5799	PaO_2 — lactate	0.5979	RBCC — MCH	—0.6049
Ht — lactate	0.5512	CaO_2 — HCO_3^-	—0.6524	CaO_2 — MCV	0.5856
Ht — Vf	0.4767	CaO_2 — Vf	0.5326	lactate — HCO_3^-	0.8692
		RBCC — MCHC	0.6759	Ht — Hb	0.5484
		MCH — VO_2	0.6088	Hb — MCHC	0.5861
				MCH — MCHC	0.5404

tions were performed. Firstly, data obtained at the different sampling times were cinsidered separately. Secondly, general correlations were calculated with all values for every variable without distinction of hour of measurement.

The results of the first analysis showing correlation coefficients for the 0, 3 and 24 h samples are given in table II. A very low number of significant correlations are evident for each sample, significant correlations being only 10-12 % from all calculated variables.

Table III shows coefficients for general correlations, asterisks indicating those which are significant. pHa is positively correlated with PaO_2 and with CaO_2 and negatively correlated with $CaCO_2$, bicarbonate and lactate. Further a positive correlation exists between PaO_2 and CaO_2 and between PaO_2 and bicarbonate is highly significant, which should be expected in view of the derivation of bicarbonate concentration from the Henderson-Hasselbach equation.

The majority of haematological parameters (Ht, Hb, MCV, MCH and

Table III. *Significative and indicative Pearson correlation coefficients for all the values obtained in the three samples.* Significant coefficients are marked with *. Abbreviations as in table I.

					MCHC	MVC	MCH	VO_2	
PaO_2	0.5746*						0.5569*		MCHC
CaO_2	0.4489*	0.6800*				0.5353*			MCH
$CaCO_2$	—0.7089*	—0.4121*							VO_2
HCO_3^-	—0.6878*	—0.4077*		0.9991*				0.4151*	Vf
lactate	—0.4061*								
Ht	—0.6790*	—0.5610*	—0.2666	0.5895*	0.5693*	0.4982*			
Hb	—0.6083*	—0.5211*		0.4240*	0.4097*	0.4888*	0.8338*		
RBCC							0.3177	0.2738	
MCV	—0.5667*			0.5053*	0.4880*		0.4195*	0.3390*	—0.6658*
MCHC	—0.3382*	—0.4209*		0.3538			0.4690*	0.8313*	
MCH	—0.4341*			0.4773*	0.4608*	0.4384*	0.4447*	0.7124*	
VO_2		—0.5057*	—0.3291				0.4003*	0.3632*	
Vf									
	pHa	PaO_2	CaO_2	$CaCO_2$	HCO_3^-	lactate	Ht	Hb	RBCC

MCHC) are positively correlated. However, no significant correlation exists between MCV and MCHC indicating that any change in red blood cell size is not associated with an increase in haemoglobin content of the individual erythrocyte.

A third group of correlations are those between haematological parameters and blood gas variables. With the exception of RBCC, haematological parameters are negatively correlated with pHa, CaO_2 and PaO_2 and positively with $PaCO_2$, bicarbonate and lactate.

Finally, VO_2 is negatively correlated with PaO_2 and CaO_2 and positively with Ht, Hb and Vf.

Discussion

Data from different sampling times were considered separately in the first analysis (table II). Such an analysis gives information about which variables are related in each sample. Significant correlations observed were only 10-12 % from all calculated variables. Similar analysis performed on *Hypophthalmychthys molitrix* obtained the same figure (8). In the present study only two of the same pairs of variables were correlated at each of the three sampling times. It is apparent, therefore, that when each sampling time is regarded individually, a certain number of correlations between pairs of parameters exist. However, when each sampling time is compared with the other two, those correlations apparent in one group do not inevitably reappear within the others. Such results suggest that during the recovery period possible interactive relationships between respiratory/haematological parameters are verifiable only along the whole time course but not at a single instant as determined by individual sampling techniques. The ability to monitor such parameters continuously during the recovery period would perhaps take into account any lag or phase differences between relationships over the time course which the present experimental technique necessarily excludes. Further, the possibility that we have failed to measure important parameters which may effect a controlling function on the integrative recovery of the physiology of the animal must not be excluded.

Second analysis considered general correlations for all values without distinction of hour of measurement thus permitting assessment of which parameters followed a parallel, and possibly interactive, evolution along the observed time course.

Results for correlations between blood gas parameters are similar to those obtained in previous works (10) for a number of freshwater species after subjection to anaesthesia, although in the present study additional stresses of handling and operational procedures may also be contributory factors. As previously concluded (4), such procedures invoke a considerable state of internal hypoxia. MS-222 is a known asphyxiant of fish (12), which inhibits ventilation and gaseous exchange across the gills. Further, MS-222 also produces an environmental hypercapnia thus influencing the carbonic buffering system of the blood (1). $CaCO_2$ and bicarbonate therefore increases. In association with the marked lactacidosis, increased CO_2 composition of the blood leads to the marked decrease in arterial pH evident after anaesthesia and operational procedures. Such an induced hypoxia, as also indicated by decreased CaO_2 and PaO_2, implies severe interference with gaseous exchange processes with a pH mediated Bohr shift probably playing a dominant role.

Increase in MCV following anaesthesia has been found in trout (12) and has been partly associated with increased haemoconcentration (6), but this phenomenon appears absent from the dogfish. Increased Ht following anaesthesia has

been also observed in trout (9). In the present study, RBCC does not significantly decline despite sequential sampling suggesting that a net increase of cell size occurs. Recruitment of red blood cells *de novo* from the spleen could lead to an increase in Ht which despite sampling does not significantly change over time. However, increased MCV would appear to be the major effect of the anaesthesia-induced hypoxia.

The existence of a negative correlation between RBCC and MCV indicates that as RBCC may decrease, MCV should increase. Although MCV increase has been regarded as a passive consequence of the anaesthesia (4), it is possible that it may be an adaptative compensation. An increased erythrocytic size may lead to increased surface area available for oxygen uptake by the haemoglobin under hypoxic conditions when oxygen is less available. Further, increased blood viscosity through erythrocytic swelling may increase residence time in the gill vasculature hence increasing time available for gaseous exchange. Such a strategy might partially compensate for the pH dictated decrease in haemoglobin oxygen affinity (Root effect) apparent in many fishes species. The very low number of erythrocytes in elasmobranchs in comparison to teleosts (3) also suggests that a mechanism of increasing MCV during hypoxia may be a more effective strategy in maintaining oxygen supply to the tissues of these animals.

Correlations between blood gas and haematological parameters can again be partially explained in terms of erythrocytic swelling. Increase in MCV with no increase in MCHC raises blood oxygen affinity by dilution of haemoglobin within the cell (11). An increased oxygen affinity would seem empirically acceptable when PaO_2 and CaO_2 decline as a consequence of the operational procedures. When PaO_2 and CaO_2 increase with time, MCV is accordingly reduced.

VO_2 relations with some other parameters can be explained in terms of hypoxia. A decreased level of blood oxygen dictates that increased oxygen is required thus accounting for the increased VO_2 immediately after the operational procedures. Although Vf did not significantly change with time (table I), ventilatory stroke volume decreases (4), hence conforming to the decreased oxygen requirement as the recovery process progresses.

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Resumen

Se ha determinado una amplia serie de parámetros hematológicos y respiratorios en 22 ejemplares de *Scyliorhinus canicula* inmediatamente después de la canulación de la aorta dorsal y después de 3 y de 24 horas. El estudio de las correlaciones entre las variables determinadas indica que el hinchamiento de los eritrocitos juega un papel importante en el mantenimiento del suministro de oxígeno a los tejidos. Los resultados se discuten en función del estrés producido por la manipulación, anestesia y cirugía y de la dinámica del proceso de recuperación.

References

1. Borjeson, H.: *J. Fish Biol.*, **11**, 133-142, 1977.
2. Cameron, J. N.: *Comp. Biochem. Physiol.*, **38A**, 699-704, 1971.
3. Duthie, G. G.: Ph. D. Thesis. University of Aberdeen, 1980.
4. Duthie, G. G. and Tort, L.: *Comp. Biochem. Physiol.*, **81** (A), 879-883, 1985.
5. Glanz, S. A.: *Circulation*, **61**, 1-7, 1980.
6. Houston, A. H., Madden, J. A., Woods, R. J. and Milles, H. M.: *J. Fish. Res. Bd. Can.*, **28**, 635-642, 1971.

7. Pickering, A. D.: In «Stress and fish» (Pickering, A. D., ed.), Academic Press, London, 1981, pp. 1-7.
8. Pieterse, J. J., Smit, G. L., Van Kliet, K., Schoonbee, H. J. and Hatting, J.: *J. Fish Biol.*, **18**, 455-459, 1981.
9. Reinitz, G. L. and Rix, J.: *Comp. Biochem. Physiol.*, **56**, 115-116, 1977.
10. Smit, G. L., Hattingh, J. and Burger, A. P.: *J. Fish Biol.*, **15**, 633-643, 1979.
11. Soivio, A. and Nikinmaa, M.: In «Stress and fish» (Pickering, A. D., ed.), Academic Press, London, 1981, pp. 247-275.
12. Soivio, A., Nyholm, K. and Huhti, M.: *J. Fish Biol.*, **10**, 91-101, 1977.
13. Soivio, A. and Oikari, A.: *J. Fish Biol.*, **8**, 397-411, 1976.
14. Soivio, A., Westman, K. and Nyholm, K.: *Finnish Fish Res.*, **1**, 11-21, 1972.
15. Tucker, V. A.: *J. Applied Physiol.*, **23**, 410-414, 1976.