An experimental study of postmortem ocular fluid and core temperature analysis in incidentally captured harbour porpoise (*Phocoena phocoena*)

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ABSTRACT

Determination of elapsed time since death in small cetaceans can be important to our understanding of the nature of their interactions with fishing operations. This pilot study was conducted to determine the potential diagnostic usefulness of ocular fluid (vitreous humour) and core body temperature to estimate postmortem intervals in harbour porpoises (*Phocoena phocoena*). Core temperature and concentrations of various constituents of vitreous humour (glucose, urea, sodium, potassium, chloride, magnesium, calcium, and phosphorus) were determined in 24 harbour porpoises incidentally caught in groundfish gillnets in the waters of the Gulf of Maine and the Bay of Fundy. These parameters were compared to published values for rectal temperatures and the serum concentrations of several selected elements in live harbour porpoises. Glucose in vitreous humour decreased in dead animals compared to serum values in live ones; its level was positively correlated with core temperature. Potassium and magnesium in vitreous humour increased following death. These data suggest that most animals analysed had been dead for several hours. For the present, the methodology affords researchers an approach that appears to hold some promise. However, the most practical technique requires testing animals with a known time of death in order to derive a set of curves for ocular fluid values and temperature versus time that are appropriate for a statistical presentation of predictability for the time since death.

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INTRODUCTION

During the past several decades concern has grown over the impact of harbour porpoise (*Phocoena phocoena*) mortality due to incidental capture in sunken gillnets as fishery effort by commercial gillnets expanded (Jefferson and Curry 1994). For harbour porpoises incidentally caught in fixed groundfish gillnets, estimation of time since death, or the postmortem interval (PMI), may provide answers to questions regarding when animals are caught during fishing operations and may facilitate identification of the reasons for capture.

Several methods are under consideration to mitigate incidental captures of harbour porpoise (Polacheck 1989, Dawson 1991, Jefferson and Curry 1994, De Conti 1996). Evaluation of the likely effectiveness of these methods suffers from a paucity of information regarding the behaviour of porpoises near the nets and the scenarios that results in capture (Lien *et al.* 1995). An important question regarding harbour porpoise captures in bottom fishing nets is whether entrapment is more likely during net deployment, while the net is fishing, or during its retrieval. Sinking and retrieval times of nets fishing at depths of 300 m or more may be in excess of 30-60 min each. Therefore, there is a significant period of time (perhaps as high as 10-15% of total fishing time) during which nets could catch porpoises at less than target depths. Porpoises may have greater difficulty detecting clean nets when they are first placed in water, or nets full of fish could attract them as these are hauled out. At present, data are insufficient to determine when in time porpoises are captured.

Determining when the porpoise is caught has important management implications, because modifying fishing methods may help to mitigate incidental captures. If captures occur as the net descends, for example, then heavier anchors, which sink the net more quickly, may minimise catches of harbour porpoises. If entanglement occurs as the net is being hauled out, then shorter strings requiring less hauling time may reduce catches. Enhancing the detectability of the net (Lien *et al.* 1995) might reduce captures which occur as the net fishes.

The use of ocular fluid (aqueous or vitreous humour) and core body temperature to estimate PMI has been discussed extensively in the literature on human forensic science (Farmer *et al.* 1985, Coe 1989, Sebag 1989, Knight 1991, Henssge *et al.* 1995), wildlife management (Johnson *et al.* 1980, Pex *et al.* 1983, Woolf and Gremillion-Smith 1983, Cox *et al.* 1994, Zaugg and Kinsel 1997), and veterinary medicine (McLaughlin and McLaughlin 1987, Henke and Demarais 1992). These studies indicate the need for caution in interpreting postmortem changes in any individual parameter and for concurrent evaluation of multiple parameters in determination of PMI. The antemortem concentrations of chemical constituents of ocular fluid are normally at equilibrium with their concentrations in serum, but the ratios of these concentrations may vary among constituents, individuals, and species (Farmer *et al.* 1985, McLaughlin and McLaughlin 1986) (Table 1). Moreover, environmental factors, particularly ambient temperature, can influence the rates of postmortem changes in these constituents (Wilkie and Bellamy 1982, Farmer *et al.* 1985, McLaughlin

Table 1: Ratios of mean concentrations of various constituents in fresh vitreous humour to their mean serum concentrations in domestic animals.

	Feline	Canine	Porcine	Bovine
Glucose[a]	0.86	0.88	---	1.13
Urea[b]	---	0.82	0.88	0.76
Sodium[b]	---	1.03	0.93	0.97
Potassium[b]	---	1.56	0.66	0.77
Magnesium[b]	---	---	1.00	1.04
Calcium[b]	---	---	0.51	0.58
Phosphorus[b]	---	---	0.09	0.15
Chloride[b]	---	1.13	1.15	1.17

[a] Ratio calculated from mean concentration of vitreous glucose provided by Hanna *et al.* (1990) and from reference values for serum glucose used at the Atlantic Veterinary College, University of Prince Edward Island, the same laboratory in which work by Hanna *et al.* (1990) was performed;
[b] McLaughlin and McLaughlin (1986).

and McLaughlin 1986) and in core body temperature (Henssge *et al.* 1995).

Farmer *et al.* (1985) observed that, in human cases of drowning, increases in concentrations of ocular magnesium and decreases in concentrations of ocular sodium were related, albeit erratically, to the length of immersion period in salt water and fresh water, respectively. Nonetheless, some postmortem changes are consistent among individuals and species: ocular glucose concentration decreases after death due to its continued utilization by autolytic processes, although the rate of decrease can be erratic and precipitous (Coe 1972); ocular potassium concentration increases after death, as this electrolyte is released from the cytoplasm of cells, particularly those of the retina, during autolysis (Coe 1989, Sebag 1989), and core body temperature decreases until it reaches ambient temperature (Knight 1991). By comparison, changes in the concentrations of other constituents of vitreous humour, such as lactate, pyruvate, ascorbate, non-protein nitrogen, sodium, chloride, phosphate, and bicarbonate have been found to be either too variable or too small to be useful in investigation of PMI (Henry and Smith 1980, Farmer *et al.* 1985).

The objectives of this study were to: (1) compare the postmortem concentrations of various components of vitreous humour (glucose, urea, sodium [Na], potassium [K], chloride [Cl], magnesium [Mg], calcium [Ca], and phosphorus [P]) in harbour porpoises incidentally caught in fishing nets with the serum concentrations of these elements in live porpoises from values published in Koopman *et al.* (1995); (2) to evaluate the relationship between the composition of vitreous humour constituents; (3) investigate specifically glucose, potassium and magnesium to core body temperature and duration of fishing time of the nets and to (4) assess the potential usefulness of these tools in determining PMI in harbour porpoises.

MATERIALS AND METHODS

Animals

Between 8 August and 7 September 1994 and between 1 July and 22 September 1995, core body temperature was measured in, and samples of vitreous humour were collected from, 24 harbour porpoises (Table 2) incidentally drawn in sunken gillnets which were approximately 1.5 fathoms in height and located off Grand Manan Island in the Bay of Fundy, Canada (44° 40' N, 66° 50' W). Mean ambient water column temperature in this region for the two seasons (collected with a Seabird SBE-19 conductivity and temperature at depth recorder; Sea Bird Electronics, Inc., Bellevue, WA, USA) was 8.6° C ± 1.9° C (range 7.0-12.6° C); depth of the gillnets was 96.6 ± 9.58 m (range 86-112 m).

Collection of samples

Animals were retrieved dead from gillnets and brought aboard the fishing vessels where they were measured for standard length in centimetres (defined as the straight line distance between the tip of the rostrum and the fluke notch in a straight line parallel to the body) and girth (cm, measured circumference of porpoise mid-point between anterior of dorsal fin and the pectoral fin). The measuring tape was held securely but not tightly, to avoid compressing the tissue. The core body temperature was taken immediately after the measurements. An incision lateral to the ventral midline and extending from the cranial insertion of the left pectoral flipper to the urogenital opening was made through the blubber layer into the abdominal cavity. The liver was identified and a thermometer (Bl-Metal 12.5 cm-stem thermometer, VMR CANLAB, Mississauga, ON, Canada) was inserted approximately 10 cm into its left aspect. The thermometer was left in place for a minimum of 3 minutes for the temperature to stabilize, and read *in situ.* Samples of vitreous humour were collected immediately following placement of the thermometer into the liver. Vitreous humour was selected over aqueous humour because of its larger volume and easier collection. Fluid was extracted from both eyes by insertion of a 16- or 20-gauge needle through the lateral canthus into the central region of the vitreous body, followed by gentle aspiration of 1-2 ml of ocular fluid into a 5-ml syringe, using a separate syringe for each eye. All animal carcasses were returned to ocean water immediately upon completion of sample collections to honor an agreement made with the fishermen that no retrieved animal would be retained and brought to shore.

Table 2: Vitreous concentrations (mmol/L) of potassium (K), magnesium (Mg), glucose, core body temperature (°C), the soak time (hours) of the nets and sex, length (cm/ranked longest to shortest), blubber thickness (cm) and girth (in cm/measured circumference of porpoise mid-point between anterior of dorsal fin and the pectoral fin) of 24 harbour porpoises drawn in gillnets in the Bay of Fundy, Canada.

Animal number	Sex	Length	Rank to length	Blubber	Girth	K	Mg	Glucose	Core temperature	Soak time of net
1	F	141.0	12	1.5	87.0	n/a	n/a	0.10	10.0	17.15
2	F	138.5	14	1.3	93.0	n/a	n/a	0.95	16.0	19.00
3	M	132.5	15	1.7	82.0	n/a	n/a	0.10	16.0	48.00
4	M	112.5	19	2.4	81.0	n/a	n/a	0.05	12.0	25.00
5	M	146.0	9	2.0	91.0	n/a	n/a	2.30	22.0	23.00
6	M	104.5	20	4.0	78.5	n/a	n/a	0.10	10.5	25.00
7	F	152.0	6	1.5	101.0	n/a	n/a	1.55	30.0	27.00
8	F	153.0	5	1.4	87.0	n/a	n/a	0.50	16.0	26.00
9	F	171.0	1	1.7	97.0	n/a	n/a	0.30	16.0	24.00
10	F	166.0	2	1.8	93.0	n/a	n/a	1.70	20.0	95.00
11	F	117.0	18	2.2	78.5	n/a	n/a	0.00	12.0	95.00
12	F	149.0	7	2.0	80.5	14.3	8.90	0.20	9.0	21.15
13	M	123.0	17	1.9	81.0	8.65	1.50	1.85	18.0	22.30
14	M	152.0	6	1.3	92.0	9.35	1.30	0.55	12.0	24.30
15	F	144.0	10	1.5	91.0	12.6	4.90	0.70	11.0	43.15
16	F	154.0	4	1.5	95.5	9.9	1.50	1.00	11.0	26.00
17	M	131.0	16	1.4	70.0	8.2	1.00	0.75	12.0	19.30
18	M	148.0	8	1.3	91.5	11.1	n/a	0.20	8.0	46.45
19	M	140.0	13	1.3	86.0	14.3	7.50	0.50	13.0	24.30
20	F	148.0	8	1.6	88.0	11.9	3.40	0.05	9.0	26.00
21	M	155.0	3	2.1	99.0	14.0	4.50	0.85	12.0	24.00
22	M	143.0	11	1.8	89.5	15.8	2.00	0.75	20.0	24.30
23	F	152.0	6	2.4	99.0	9.55	1.40	2.10	21.0	21.00
24	M	96.5	21	3.0	74.0	18.8	1.80	0.00	10.0	69.15

Nine animals had one eye with advanced deterioration (which appeared to be due to scavengers) making collection of fluid from both eyes impossible. Samples were kept in vials placed in plastic bags then into protective containers away from direct sunlight at ambient air temperature (11-20^{0}C). Upon return to shore they were immediately centrifuged for 10 minutes at 2,000 rpm and the supernatant frozen and kept at –20° C until analysis.

Analysis of samples

Core body temperature and concentrations of vitreous glucose, urea, Na, Cl, and P were determined in all animals retrieved in 1994 and 1995. These data were pooled for analysis. In 1995, concentrations of vitreous K, Mg and Ca were also determined (these constituents were not collected in 1994). All analyses of the vitreous elements were done with a BM/Hitachi 911 blood chemistry multianalyser (Boehringer Mannheim Corporation, Indianapolis, IN, USA). Postmortem core body temperatures were compared to the mean rectal temperature of 36.2° C in 2 (one juvenile, one adult) normal captive harbour porpoises (R.A. Kastelein, Harderwijk Marine Mammal Park, The Netherlands, 1994, pers. comm.). No information is

Table 3: Concentrations of various constituents of ocular vitreous humour in harbour porpoises drawn in gillnets in the Bay of Fundy, Canada, compared to serum concentrations of these constituents in harbour porpoises caught and released live from herring nets in the same region (Koopman *et al.* 1995). In all cases, the gillnets had been in water for >17 h. All values are in mmol/L.

Constituent	n	Vitreous humor (mean ± s.d.)	Range	Serum (mean ± s.d.)[a]	
Glucose	24	0.71± 0.70	0 – 2.3	10.87 ± 1.46	*
Urea	24	15.64 ± 4.94	3.0 – 22	21.14 ± 4.33	
Sodium	24	197.0 ± 59.05	140 – 349	156.6 ± 7.7	
Chloride	24	166.0 ± 69.34	103 – 354	114.3 ± 3.8	
Phosphorus	24	1.54 ± 1.35	0.40 – 5.62	1.76 ± 0.60	
Potassium	13	12.18 ± 3.15	8.2 – 18.8	4.64 ± 1.30	*
Magnesium	12	3.30 ± 2.63	1.00 – 8.9	0.75 ± 0.16	*
Calcium	13	2.86 ± 1.74	1.72 – 8.2	2.41± 0.16	

* indicates a significant differnce (P<0.05) between the serum and vitreous humor means

[a] Koopman *et al.* (1995).

available on the concentrations of the various constituents of normal vitreous humour in freshly dead harbour porpoises. Postmortem concentrations of vitreous constituents were compared to serum concentrations from live harbour porpoises incidentally captured and released from herring weirs in the Bay of Fundy (Koopman *et al.* 1995) and, thus, assumed to belong to the same population as the animals sampled in the current study.

Data analysis

A paired, 2-tailed t test was used to determine if there were differences between the concentrations of elements in fluid extracted from individual eyes of a single animal. Concentrations of each element in individual eyes of the same animal did not differ from the mean concentration for both eyes (P>0.05). Subsequently, for further statistical analysis, the mean concentrations of chemicals in both eyes (when present) were used. All associations were examined using correlation coefficient and linear regression analyses. Correlation coefficients were compared using R tests. Slopes of various regression lines were compared using t tests. Tests were considered significant if P<0.05. Statistical procedures were performed using SigmaStat Scientific Software (Jandel Corporation, San Raphael, CA, USA).

RESULTS

The mean (± s.d.) soak time of gillnets from which the 24 animals were retrieved was 34 h ± 22 h, with a range of 17.15 to 95 h. The mean (± s.d.) postmortem core temperature of these 24 animals was 14.6 ± 5.2° C, with a range of 8-30° C (Table 2). There was a significant difference (P<0.001) between serum concentrations and postmortem vitreous concentrations of glucose, K, and Mg (Table 3, Figure 1). Glucose concentration in all samples of vitreous humour decreased to less than 25% of the antemortem serum concentrations. There was a significant positive correlation between this concentration and core body temperature (R=0.72; P<0.0001) and a slight negative correlation between it and the vitreous concentration of K (R=0.54; P<0.05). There was no statistically significant correlation between the vitreous concentration of glucose and the soak time of gillnets and the vitreous concentrations of Na, Mg, Ca, P, Cl, and urea (P>0.05).

Two animals (#11 and #24) had no detectable glucose in their vitreous humour and a core temperature of <13° C; the soak times of the gillnets in which these 2 animals had been caught were 95 and 69 h, respectively (Table 2).

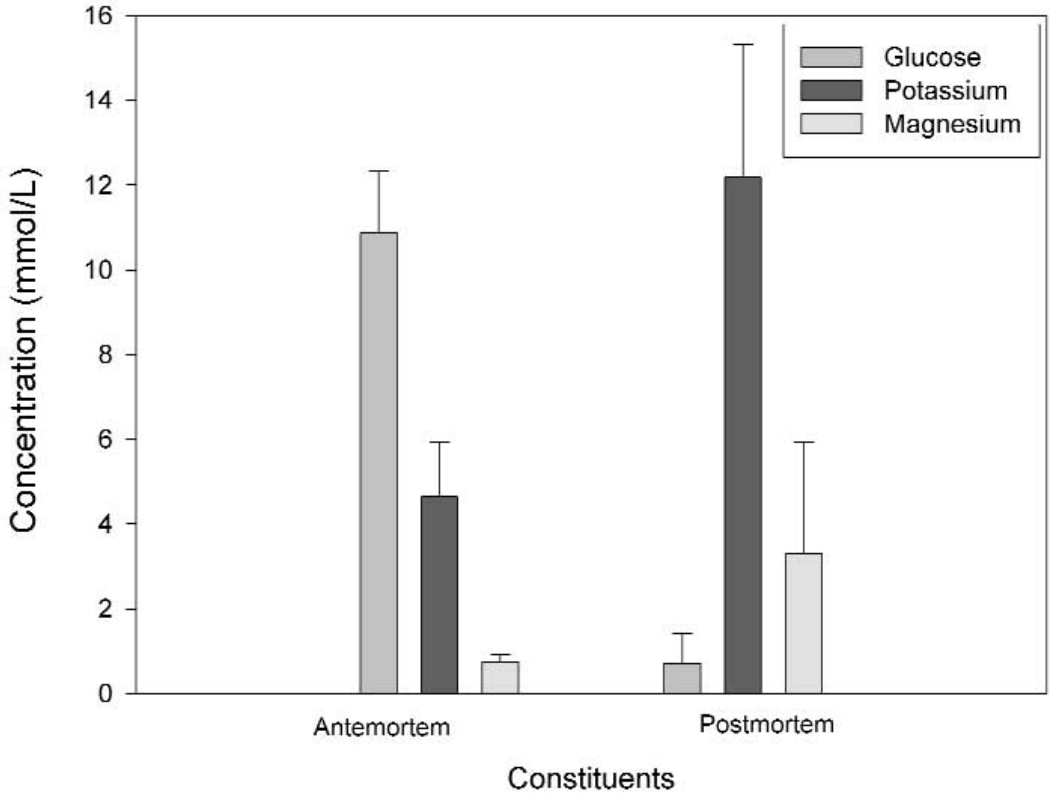

Fig. 1: *Comparison of mean concentrations of potassium, magnesium, and glucose in serum of harbour porpoises incidentally captured and released from herring weirs and in vitreous humour of harbour porpoises incidentally drawn in gillnets. Antemortem serum values are from Koopman et al. (1995).*

Conversely, the 2 animals (#5 and #23) with the highest vitreous concentration of glucose (>2 mmol/L) had among the highest core temperatures (>20° C), and the gillnets in which they had been caught had some of the shortest soak times (23 and 21 h, respectively) (Table 2). However, one animal (#10) with a relatively high vitreous concentration of glucose (1.7 mmol/L) and a 20° C body temperature had been caught in a gillnet with a soak time of 95 h (Table 2). Ten of 13 animals with a 90% or greater decrease in vitreous concentration of glucose, as compared to serum concentrations displayed a >100% increase in vitreous concentration of K. Seven of 12 animals with a >90% decrease in vitreous concentration of glucose had a >100% increase in vitreous concentration of Mg.

Weak but noteworthy relationships were found between the vitreous concentrations of K and P (R=0.62; P<0.05) and between the vitreous concentration of K and the soak time of gillnets (R=0.54; P<0.05). The vitreous concentration of K was not correlated in a statistically significant manner with either the core temperature or the vitreous concentrations of Na, Mg, Ca, Cl, and urea (P>0.05). Animal #24, a very young animal with the shortest length and one of the thickest blubbers, also had the highest vitreous concentration of K and no detectable glucose. Its core body temperature was 10° C, but the concentration of Mg in its vitreous humour was only 1.80 mmol/L. The gillnet in which it had been caught had a soak time of 69 h (Table 2). In contrast, of the 12 animals for which the vitreous concentrations of K and Mg were determined, animal #17 had the lowest concentrations of both electrolytes, and the gillnet in which it had been caught had one of the shortest soak times (19.30 h) (Table 2). Yet, the core temperature of this animal had already reached 12° C.

Strong positive correlations were found between the vitreous concentration of Mg and those of Na (R=0.97; P=0.0001), Cl (R=0.97; P=0.0001), and Ca (R=0.97; P=0.0001 Figure 2). The vitreous concentration of Mg was not correlated in a statistically significant manner with either the soak time of the nets, the core temperature, or the vitreous concentrations of glucose, urea,

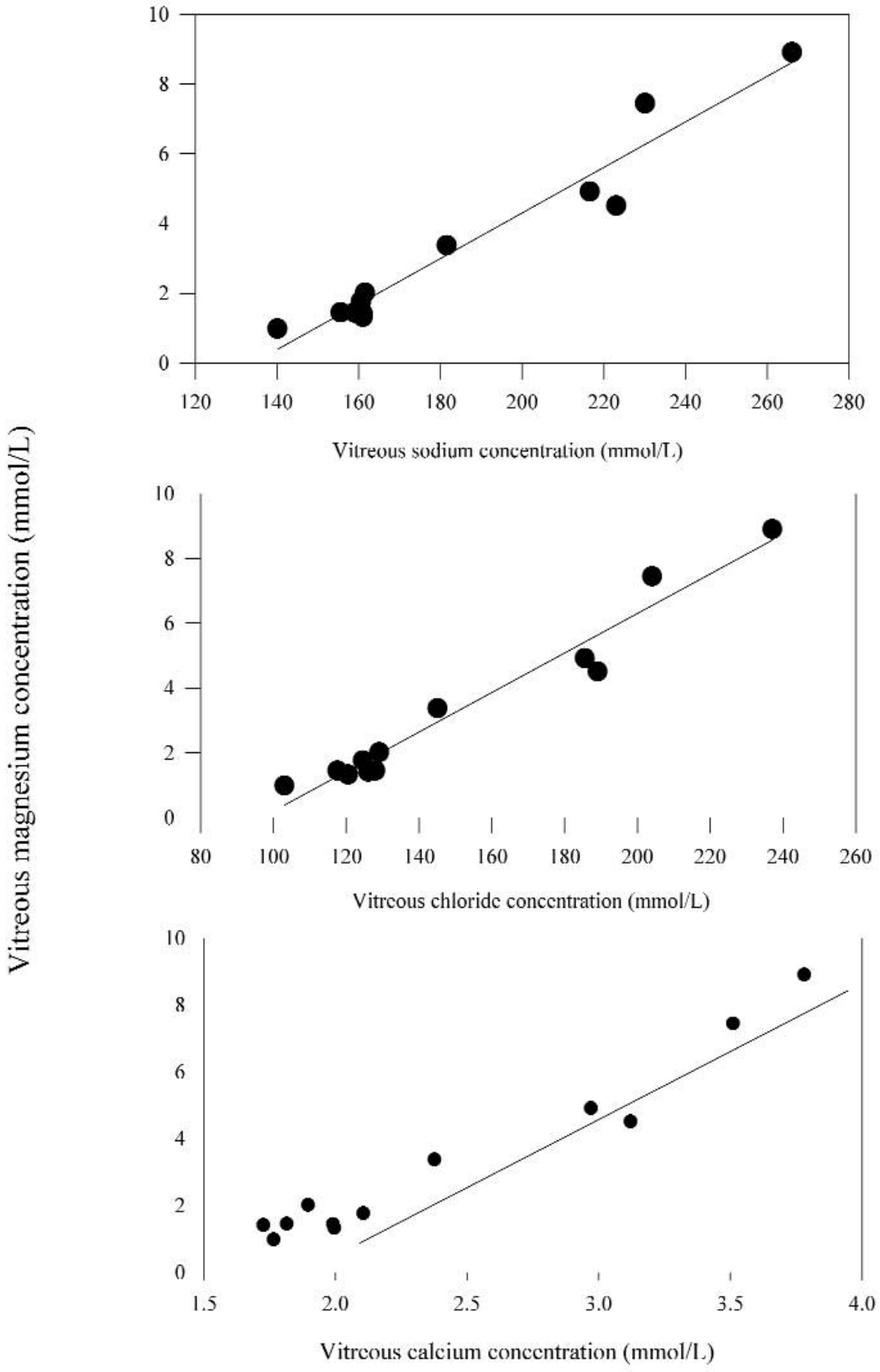

Fig. 2: *Comparison of mean concentration of magnesium to sodium (R=0.97; P< 0.0001), chloride (R=0.97; P< 0.0001) and calcium (R=0.97; P< 0.0001) values (mmol/L) in vitreous humour of harbour porpoises that were incidentally drawn in gillnets. The regression line is at the 99% confidence interval.*

Table 4: Summary of vitreous humour and core temperature correlations. Variants include glucose, potassium (K), magnesium (Mg), and postmortem core temperature examined for correlation with ancillary elements.

Variant	Glucose	K	Mg	Core temperature
Calcium			****	
Chloride		*	****	
Glucose				****
Magnesium				
Phosphorus		*		
Potassium	*			
Sodium			****	
Sodium/Potassium ratio		****		
Urea				
Girth				*
Soak time		*		

Note: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$

P, and K ($P > 0.05$). However, animal #12 had the highest vitreous concentration of Mg, one of the highest vitreous concentrations of K, and one of the lowest vitreous concentrations of glucose. Its core temperature was 9° C, but the gillnet in which it had been caught had a soak time of only 21 h (Table 2). Data analyses for vitreous humour constituents are summarised in Table 4.

DISCUSSION

Many authors have warned about the difficulty of using changes in concentration of various constituents of vitreous humour for estimating PMI in animals and humans (Coe 1972, 1989; Knight 1991, Henssge *et al.* 1995). Variations in antemortem concentrations of these constituents among normal individuals, variations in the intrinsic rate of change of these concentrations following death, and the influence of different environmental conditions, particularly ambient temperature, on the autolytic process combine to complicate predictions of PMI based on analysis of these constituents. In addition, if haemorrhaging occurs in the ocular fluid due to pressure from the net, the value of the parameter measured may be influenced. For this study samples were collected under careful protocol and no blood was detected during fluid analysis. Because of these factors, it is essential to evaluate more than one parameter from an individual in order to avoid being misled by potential erratic changes in any single parameter. The postmortem changes in vitreous concentrations of glucose, K, and Mg observed in this study show trends similar to those found in other studies in animals and humans, whereby concentrations of glucose decrease and those of K increase after death, while concentrations of Mg increase with immersion time in salt water (Coe 1989, Farmer *et al.* 1985, Knight 1991, Henssge *et al.* 1995). However, in some of the animals examined in this study, the magnitude of postmortem change was not uniform among the various constituents of vitreous humour analysed, thus demonstrating the inconsistent nature of this process.

Postmortem decrease in body temperature and changes in concentrations of some vitreous constituents tend to reach an equilibrium rapidly and, therefore, may be of limited use in estimation of PMI when the latter is more than 1-2 days. McLellan *et al.* (1995) observed that a harbour porpoise dead for less than 10 min had a colonic temperature of about 33° C in an ambi-

ent air temperature of 14-16° C. Following return to sea water of approximately 13° C, the core body temperature (measured by insertion of a thermometer in the epaxial muscle mass) decreased gradually for 500 min (8.3 h) at an average rate of 2.5° C per hour until it reached ambient water temperature. After 24 h, there was no evidence that the temperature of the carcass had increased as a result of putrefaction. Based on their results, McLellan *et al.* (1995) proposed that any similarly sized carcass with above ambient intramuscular temperatures died within the last 8-10 h. Similarly, a carcass at approximately 20° C died within the past 6 h; 30° C within the past hour. According to these calculations porpoise number 7 is the only porpoise possibly captured during the hauling of the net. The results of the present study can neither support nor refute those of McLellan *et al.* (1995), since the exact time of death of the animals was unknown and since the ambient water was substantially colder. In addition, body temperature may vary between individuals due to different levels of stress at the time of death (Henssge *et al.* 1995).

Cooling time is a function of the physical characteristics of the body and the insulation surrounding it. A variety of factors such as age, body mass, depth of blubber, girth size and body surface area effect the rate of postmortem cooling. For example animals 4, 6 and 24 were the shortest in length and had the deepest blubber layers present indicating they were the youngest animals captured. These factors may affect cooling rates because of the insulative effect of blubber along with the body volume relative to length, which is greater in juveniles than in adults. When calculating cooling rates in harbour porpoise these factors must be considered and a standardised protocol for obtaining body cooling rates established. For this study core body temperatures were chosen for the following reasons.

When death occurs, circulation, the mechanism for transferring heat from the inner core to the surface, stops, causing heat to immediately start flowing from the surface to the surrounding environment. Concurrently, temperature within the inner core organs including the liver remains constant for a period of time and will not commence decreasing until a heat gradient is established between the core of the body and the surface (Henssge *et al.* 1995). Core temperatures are considered to be more accurate than rectal values as the core temperature is closer to the centre of the body and is thought to be more uniform for a greater period of time after death. Additionally, use of core temperature permits selection of a standard location for measurement of temperature thus increasing accuracy (Knight 1991).

According to Coe (1972), vitreous concentrations of glucose can show precipitous drops to very low levels in a matter of a few hours in some human cadavers. In the present study, the vitreous concentration of glucose in all porpoises examined decreased to less than 25% of the antemortem serum concentration as determined by Koopman *et al.* (1995). The range of serum glucose concentrations obtained from animals sampled by these authors (8.2-13.8 mmol/L) was higher than that reported by Bossart and Dierauf (1990) (3.3-7.8 mmol/L). Animals examined by Koopman *et al.* (1995) were considered stressed because they had been caught in fishing weirs and, therefore, may have experienced hyperglycemia due to elevated glucocorticoid activity. Similarly, entrapment in gillnets likely places the animal under extreme stress and presumably causes terminal hyperglycemia. However, it is doubtful that there would be enough time prior to death for the elevated serum glucose concentrations to equilibrate with those of the vitreous humour. Nonetheless, for the majority of porpoises, the decrease in vitreous concentration of glucose was so dramatic that the difference with serum concentrations would have remained highly significant even with the use of lower serum values for comparison.

According to Adjutantis and Coutselinis (1972), the increase in vitreous concentration of K in human cadavers reaches a limit determined by the K supplies that can diffuse into the vitreous body from the surrounding tissues. They suggested that this limit is about 12-13 mmol/L, that the time required to obtain such a value is about 12 h from the time of death, and that the increase of K values is linear during the first 12 h after death. In contrast, according to Henry

and Smith (1980), the postmortem vitreous concentration of K in human cadavers rises more rapidly in the first 6-12 h, but in a fairly linear fashion after 24 h, reaching a maximum of 25-40 mmol/L at 100-120 h. In cattle, the mean vitreous concentration of K increased from 4.58 mmol/L at 0 h to 7.35 mmol/L at 24 h (Hanna *et al.* 1990). Our results include a maximum vitreous concentration of K of 18.8 mmol/L (animal #24).

Vitreous concentrations of Mg are fairly stable after death in humans (Henry and Smith 1980). However, following drowning in seawater, Mg gradually diffuses from water into the eye. The human intraocular Mg content (normally 0.8-1.2 mmol/L) reaches equilibrium with the surrounding water (41.1-82.3 mmol/L) after approximately 24 h (Adjutantis and Coutselinis 1974, Henry and Smith 1980). The maximum vitreous concentration of Mg reached in our harbour porpoises was only 8.9 mmol/L, in that particular case, (animal #12) after a soak time of the net of 21 h and when the core temperature of the carcass had reached ambient water temperature. Low water temperature may have slowed the rate of diffusion of this electrolyte from water into the porpoises eyes. Perhaps more importantly, cetacean eyes have a very thick sclera which prevents its deformation under increased water pressure (Pardue *et al.* 1993) and may also act as an insulator to prevent excessive heat loss (Kastelein *et al.* 1990, Mobley and Helweg 1990). Therefore, diffusion of Mg from water into cetacean eyes may require a longer PMI than in terrestrial mammals before reaching equilibrium.

We acknowledge that keeping the samples of ocular fluid at air temperature until completion of the fishing effort for the day may have contributed to some changes in their constituents. It would have been preferable to chill these samples in ice immediately after collection in order to slow down any further decrease in glucose concentration or increase in K concentration (Bray 1983). Moreover, filtration of these samples through a disposable filter with a pore size of a few microns or less (of a type designed to attach directly to a syringe) (Wilkie and Bellamy 1982) would have further reduced the possibility of a rise in K concentration resulting from lysis of cells that may have contaminated these samples.

Whereas it seems difficult to accurately determine PMI in individual cases, some questions may be better answered by pooling data from several animals, thus circumventing the uncertainties of natural biological variation. Such is the case with this study, the main objective of which was to conduct a pilot investigation to determine if vitreous fluid and body temperature are useful diagnostic tools for estimating the time of death and concurrently the time at which harbour porpoises are most likely to get caught in a fishing net in the interval between its deployment and retrieval. Moreover, the spatial and temporal homogeneity of ambient water temperature leads to a much more uniform influence on autolytic processes than would be encountered in a terrestrial environment.

Results from most animals in this study indicate that entanglement occurs most often as the net is deployed or fishing, rather than when it is hauled out. Nonetheless, conclusions that can be derived from this research are limited, because of the small number of animals involved and because there was no opportunity to determine a sequential correlation between PMI and any of the parameters examined, including core body temperature. Future work should aim at increasing sample size and implementing a calibration study on electrolyte concentrations changes relative to time since death, recording the decrease in body temperature and gradual change in concentrations of various constituents of vitreous humour at a set ambient temperature in harbour porpoises for which the exact time of death is known. This will require multiple temperature observations and vitreous humour extractions at hourly or set time intervals. This approach will derive a set of curves for ocular fluid values and temperature decline versus time that are appropriate for a statistical presentation of predictability for the time since death.

In addition, studies should be conducted to further investigate possible relationships between the primary and ancillary ocular fluids examined for this study with an expansion to look at their role in relationship to body state. For exam-

ple the large individual variation observed in the urea values may be related to the elapsed time from the last meal. Such variation confirms the need to conduct a comprehensive examination of any parameter which may influence the values of the components being investigated. One example, as stated, would be to investigate the urea level as it relates to stomach contents, as urea values may vary depending on whether the animal has eaten before the entanglement or not.

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