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SHORT COMMUNICATION

MEASUREMENT OF VENTILATION VOLUME IN SWIMMING TUNAS

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In 1938, Van Dam obtained the first direct measurements of ventilation volume ($\dot{V}_G$) in restrained fish by using a rubber membrane, stretched around the fish's head, to separate inspired and expired water. In the ensuing 50 years, modifications of this technique have been widely applied in studies of fish physiology (e.g. Davis and Cameron, 1971). Current methods for measurement of $\dot{V}_G$ in freely moving fish (Saunders, 1962; Holeton and Randall, 1967) are indirect and require accurate determination of inspired ($P_{I_{O_2}}$) and expired ($P_{E_{O_2}}$) oxygen tensions, and oxygen consumption ($\dot{V}_{O_2}$). While $P_{I_{O_2}}$ can be easily determined, measurement of $P_{E_{O_2}}$ can be troublesome.

Studies by Davis and Watters (1970) have shown $P_{E_{O_2}}$ measurements from catheters inserted through the operculum to be position-sensitive and, therefore, extremely variable. Some authors (Kiceniuk and Jones, 1977) have tried to circumvent this problem in swimming fish by sewing a rubber 'skirt' between the mouth and opercula to ensure mixing of expired water and to separate it from the surrounding water. Even when expired water P_{O_2} can be determined accurately, the measurement of $\dot{V}_{O_2}$ of fish swimming in a large volume of water is next to impossible.

An alternative method has been described for direct measurement of $\dot{V}_G$ in restrained fish by using a bolus injection (i.e. Hamilton–Stewart) dye-dilution technique (Robin *et al.* 1965; Millen *et al.* 1966), but it is not practical for use with swimming animals. In this report, we describe a continuous infusion dye-dilution technique suitable for direct measurement of $\dot{V}_G$ in swimming fish. We use this technique to measure $\dot{V}_G$ in swimming kawakawa (*Euthynnus affinis*), yellowfin tuna (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*).

A number of constraints are attached to any dye dilution method: (1) that the dye be completely mixed in the system before sampling and (2) that the dye be

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readily detectable after dilution. Fluorescein dye fulfills the latter requirement since it is accurately detectable, spectrophotometrically, at extremely high dilutions (i.e. extremely low concentrations; Fig. 1A). It also has the advantages of being harmless to the animal, noncarcinogenic (other dyes such as Evans Blue are suspected carcinogens), and inexpensive.

To mix the dye with inhalant water, a ball (approx. 0.5 cm in diameter) was blown while heating the closed end of a piece of PE240 polyethylene tubing (i.d.=1.67 mm; o.d.=2.42 mm). A range of these balls was made with various degrees of eccentricity to the side arm. The ball was perforated all over with an entomological pin. To test the effectiveness of dye dispersion over its whole surface, the ball (suspended in a 250 ml beaker of water) was connected to the battery-powered infusion pump which injected concentrated dye solution at a constant rate of approximately 10 ml min^{-1}. The ball had to be completely filled with fluid (i.e. no air bubbles) before dye injection for even dispersion of the dye.

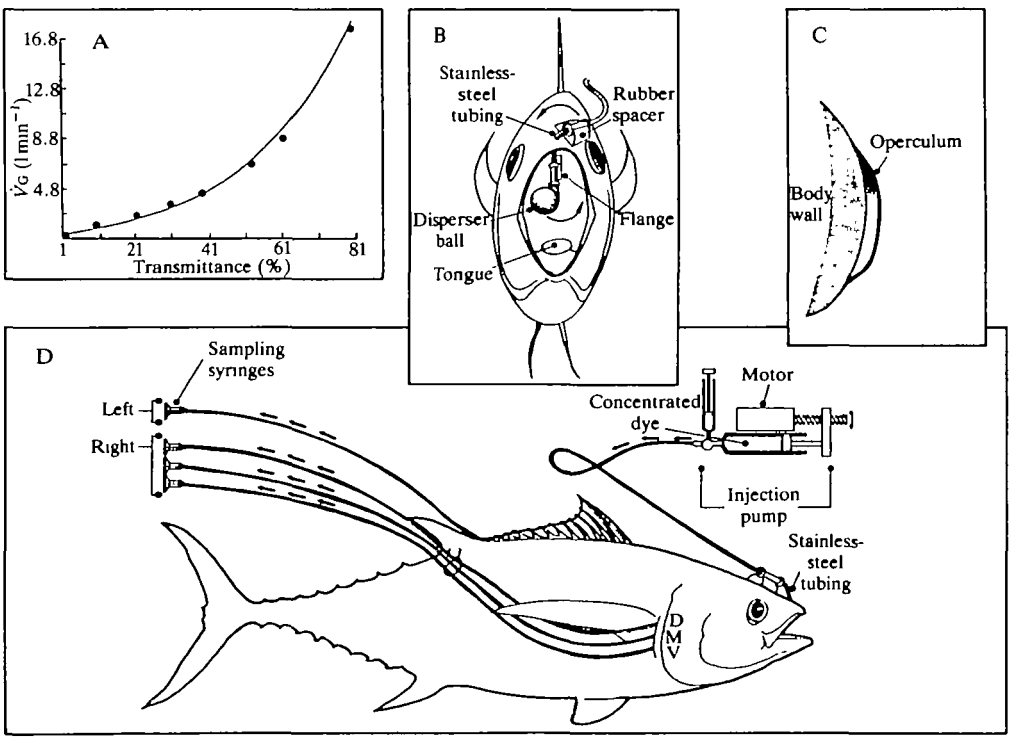

Fig. 1. (A) Dye-dilution calibration curve used to calculate $\dot{V}_G$ (l min^{-1}) from the percent transmittance of the dye sample measured spectrophotometrically. (B) Lateral adjustment of the dye-disperser ball in the buccal cavity of a tuna. (Drawn from a photograph taken through the open mouth.) Moving the stainless-steel tube in the direction of the arrow would move the disperser ball towards the midline of the mouth. (C) Diagram of the gap between the operculum and body wall when both opercula were held in the closed position. (D) Yellowfin tuna instrumented for measurement of ventilation volume. D, dorsal; M, medial; V, ventral.

A stock dye solution was made from 3–6 litres of 2 μm filtered sea water. An unmeasured amount of fluorescein dye was added until the solution became a dark rust colour. Serial dilutions (100:1, 200:1, and so on) were made, and the transmittance measured at 496 nm on a spectrophotometer. In most cases, stock dye solution concentration was adjusted, and serial dilutions remade and remeasured, until the range of expected $\dot{V}_G$ values corresponded to an exhaled dye concentration yielding approximately 20–60 % transmittance when the dye was infused at 10 ml min^{-1}. This ensured that transmittance measurements would fall on the flattest portion of the $\dot{V}_G$/transmittance curve (Fig. 1A).

To attach the dye-delivery system and disperser ball, a fish was first anaesthetized by using techniques described in Jones *et al.* (1986). After the fish had been placed upright in the operating cradle, an 18-gauge hypodermic needle was forced through the snout, in the midline, at a point midway between the inhalant and exhalant nares. The needle was removed and a blunt-ended stainless-steel tube (1.6 mm o.d.), bent at right angles, was pushed through the hole (Fig. 1B). The portion of this tubing projecting into the mouth had a ridge attached along its length, on one side. A section cut from the polyethylene tubing (which formed the side-arm of the disperser ball) locked around this metal ridge. This arrangement prevented the ball from twisting and changing its position.

The disperser ball's dorsal–ventral position was adjusted by placing a wedge-shaped rubber spacer between the fish's head and the stainless-steel tubing, so that the disperser ball occupied a midposition between the floor and roof of the buccal cavity (Fig. 1B). The cross-sectional area of the ball represented approximately 10–15 % of the area of the fish's mouth. The ball's lateral position significantly affected dye mixing. Dye concentrations were therefore measured in the exhalant water while the fish remained lightly anaesthetized and was force ventilated. If dye concentrations in the water leaving the opercula were uneven, the lateral position of the ball was adjusted by rotating the arm of the stainless-steel tubing protruding above the dorsal surface of the head (Fig. 1B). Since the disperser ball was eccentric to the stainless-steel tubing, rotating the arm adjusted the lateral position of the ball in the mouth. Dye concentrations were then remeasured. Once a suitable position was found, the stainless-steel tubing and rubber spacer were sutured tightly to the skin.

To position the 1 m long PE 90 tubing (i.d.=0.86 mm; o.d.=1.27 mm) exhalant water-sampling catheters, a 13- or 15-gauge hypodermic needle was pushed through the skin at the posterior margin of the opercular cavity, and the non-flared end of the catheter was threaded down it, from inside the cavity to the outside. The needle was withdrawn, and the catheter pulled outside the opercular cavity until the flared end of catheter lay flat against the body wall. Owing to concern about sampling from the boundary layer, PE 160 collars (1–2 mm long) were threaded over the sampling catheters in some fish before they were inserted. The collars prevented the catheters from being pulled flush against the skin and, therefore, they projected into the gill cavity. As far as we could tell, similar results were obtained from catheters with or without collars.

Table 1. *Ventilation volume and swimming speed of three species of tunas*

Tuna species	Run no.	Catheter position Side	D, M or V	Mass (kg)	L (cm)	P_{IO_2} (kPa)	P_{EO_2} (kPa)	Extraction (%)	Transmittance (%)	Ventilation volume ($l\,min^{-1}$)	Ventilation volume ($l\,min^{-1}\,kg^{-1}$)	$\dot{V}_{O_2}$ ($ml\,kg^{-1}\,h^{-1}$)	Swimming speed ($cm\,s^{-1}$)	Swimming speed ($L\,s^{-1}$)
Kawakawa-7	1	L	M	2.03	46.3	18.45	6.84	62.9	78.0	12.0	5.9	1521.8	104.2	2.3
		R	M				9.96	46.0	84.0	14.9	7.3	1380.5		
							(8.33)	(54.4)	(81.0)	(13.4)	(6.6)	(1467.2)	(104.2)	(2.3)
	2	L	M	2.03	46.3	18.45	11.09	39.9	89.1	17.9	8.8	1436.6	102.6	2.2
		R	M				11.05	40.1	90.8	19.0	9.4	1535.3		
							(11.07)	(40.0)	(90.0)	(18.4)	(9.1)	(1485.1)	(102.6)	(2.2)
Kawakawa-11	1	L	M	1.83	45.2	19.81	8.16	58.8	48.5	4.0	2.2	529.3	54.0	1.2
		R	D				6.07	69.4	47.0	3.8	2.1	591.7		
		R	M				7.35	62.9	46.3	3.7	2.0	523.3		
		R	V				9.72	50.9	44.1	3.4	1.9	391.5		
							(7.83)	(60.6)	(46.5)	(3.7)	(2.0)	(506.4)	(54.0)	(1.2)
	2	L	M	1.83	45.2	20.41	9.52	53.4	48.1	4.0	2.2	473.4	73.0	1.6
		R	D				5.56	72.8	44.9	3.5	1.9	575.5		
		R	M				6.36	68.8	44.9	3.5	1.9	544.5		
		R	V				13.00	36.3	43.9	3.4	1.9	277.1		
							(8.61)	(57.8)	(45.4)	(3.6)	(2.0)	(466.4)	(73.0)	(1.6)
	4	L	M	1.83	45.2	18.89	9.17	51.4	45.0	3.5	1.9	408.4	84.0	1.9
		R	D				5.13	72.8	45.6	3.6	2.0	590.7		
		R	M				8.33	55.9	44.0	3.4	1.9	428.0		
		R	V				10.57	44.0	42.7	3.3	1.8	321.8		
							(8.31)	(56.0)	(44.3)	(3.5)	(1.9)	(434.3)	(84.0)	(1.9)
Kawakawa-14	1	L	M	1.38	40.5	19.23	10.52	45.3	50.9	4.4	3.2	593.1	71.0	1.8
		R	D				7.56	60.7	54.0	4.9	3.6	888.2		
		R	M				8.87	53.9	52.5	4.7	3.4	747.4		
		R	V				9.07	52.8	51.9	4.6	3.3	717.3		
							(9.01)	(53.2)	(52.3)	(4.6)	(3.4)	(733.0)	(71.0)	(1.8)

	4	L	M	1.38	40.5	17.97	8.21	54.3	30.7	2.1	1.5	344.4	46.5	1.1
		R	D				5.64	68.6	30.0	2.1	1.5	424.4		
		R	M				7.71	57.1	27.8	1.9	1.4	392.2		
		R	V				7.44	58.6	30.1	2.1	1.5	363.8		
							(7.19)	(60.0)	(29.7)	(2.1)	(1.5)	(381.9)	(46.5)	(1.1)
Kawakawa-19	1	L	M	1.69	45	–	–	–	58.5	4.5	2.7	–	92.5	2.1
		R	M				–	–	62.0	5.1	3.0	–		
							–	–	(60.3)	(4.8)	(2.8)	–	(92.5)	(2.1)
	2	L	M	1.69	45	–	–	–	56.3	4.1	2.5	–	–	–
		R	M				–	–	62.1	5.1	3.0	–	–	–
							–	–	(59.2)	(4.6)	(2.7)	–	–	–
	3	L	M	1.69	45	–	–	–	60.9	4.9	2.9	–	–	–
		R	M				–	–	65.1	5.7	3.4	–	–	–
							–	–	(63.0)	(5.2)	(3.1)	–	–	–
Yellowfin-2	1	L	D	1.01	39.5	20.67	13.07	36.8	20.6	4.8	4.7	711.8	72.5	1.8
			V				9.33	54.8	19.6	4.6	4.6	1027.4		
		R	D				8.44	59.4	19.7	4.6	4.6	1115.7		
			V				9.60	53.5	20.9	4.8	4.8	1046.6		
							(10.11)	(51.1)	(20.2)	(4.7)	(4.7)	(976.8)	(72.5)	(1.8)
	2	L	D	1.01	39.5	20.00	7.60	62.0	17.0	4.2	4.2	1067.5	96.7	1.3
			V				8.93	55.3	17.4	4.3	4.3	965.2		
		R	D				8.40	58.0	19.8	4.7	4.6	1093.8		
			V				8.53	57.3	19.2	4.6	4.5	1060.3		
							(8.37)	(58.2)	(18.4)	(4.4)	(4.4)	(1046.4)	(96.7)	(1.3)
Yellowfin-3	1	L	M	1.08	38	19.53	7.59	61.2	26.3	2.7	2.5	619.1	86.5	2.3
		R	M				8.35	57.3	24.4	2.5	2.3	542.3		
							(7.97)	(59.2)	(25.4)	(2.6)	(2.4)	(579.7)	(86.5)	(2.3)
Skipjack-5	1	L	M	1.72	49	18.75	8.71	53.6	62.0	6.8	4.0	867.2	94.5	1.9
		R	M				9.41	49.8	59.8	6.3	3.7	745.0		
							(9.07)	(51.7)	(60.9)	(6.5)	(3.8)	(804.3)	(94.5)	(1.9)

The average value for each run is in parentheses.
D, dorsal; M, medial; V, ventral; *L*, body length.

The catheter was clamped in position by force threading a 1–2 mm length of PE 160 tubing (i.d.=1.14 mm; o.d.=1.27 mm), with a flared end, over it until it lay flat along the wall where the opercular catheter (Fig. 1D) exited. All catheters were sutured to the body wall just posterior to the trailing edge of the first dorsal fin. One to three catheters were placed on the fish's right side, and one catheter was on the left side in the middle position. The catheters trailed behind the swimming fish.

Finally, the fish was removed from the operating table and ventilated with sea water in a doughnut-shaped tank (17.1 m=i.d.; 17.8 m=o.d.). Fresh sea water (P_{O_2}=2.67 kPa) was pumped from a well, aerated, and fed into the doughnut tank at about 300 l min^{-1}. Depending on the demand from other users and the efficiency of the aeration system, P_{O_2} of the inflow water varied from 17.3 to 20 kPa. After recovery, the fish swam laps in the channel formed by the tank's outer and inner walls. Lap times were measured using a stopwatch.

The flow pattern of the exhalant water was established by taking photographs (using high-speed film, Kodak ASA 1000) of the fish during dye injection, as it swam by a mirror set at an angle in the swimming channel. The mirror was adjusted to obtain a ventral view along with one lateral view of the fish. Photographs confirmed that the dye exited only along the lateral sides of the fish and that none appeared from the ventro-anterior opercular edge which projects anteriorly to within 1–2 cm of the tip of the lower jaw. This was not surprising, because the inside edge of the opercular opening has a well-developed ridge and cartilaginous flap which, at least in lightly anaesthetized animals, completely closes this region to water passage. Furthermore, in tuna, the structure of the posterior margin of the operculum is not flat, but instead bows out laterally from the side of the fish (Fig. 1C). This forms a long thin orifice which allows a path for water to pass from the gills even when the opercula are pulled tight against the body wall. In practice, the opercular catheters were located to sample from the middle and/or top and bottom of the orifice.

To measure $\dot{V}_G$, two of us (one injecting, one sampling) walked around the tank, keeping up with the fish. The catheters were caught, and the disperser ball catheter connected to a portable infusion pump which was calibrated daily. Opercular catheters were connected to sampling syringes. Dye injection was started and dye sampling commenced when a steady dye stream was seen leaving the opercula (approximately 10 s). Sampling continued for 40–50 s, during which lap times were also noted. Dye concentrations were measured spectrophotometrically, and oxygen tension was measured polarographically.

In theory, if the dye had completely mixed in the mouth, the transmittance of all the samples from a single $\dot{V}_G$ measurement should have been identical. As this was never seen in practice (Table 1) we rejected data from fishes whose transmittances did not fall within ±5 % of the mean of the transmittance measured for that particular run. This resulted in the elimination of three individuals, leaving a sample size of four kawakawa, two yellowfin, and one skipjack. A statistical analysis (ANOVA) of transmittance showed no differences with respect to side

(right or left) and position (dorsal, medial, ventral) of the catheters (F=1.11, d.f.=2). To determine $\dot{V}_G$, transmittances for each trial were averaged and the average transmittance was used in the equation describing the exponential calibration curve of $\dot{V}_G$ *versus* transmittance (Fig. 1A).

As ram ventilators, tuna can enhance $\dot{V}_G$ by increasing swimming speed and/or mouth gape. In our study there appeared to be no relationship between swimming speed and $\dot{V}_G$. Therefore, we assume that other unrecorded changes, such as an increase or decrease in gape, were also instrumental in determining $\dot{V}_G$.

In some fish, oxygen extraction (%) varied markedly with the position of the sampling catheter and percent extraction determined from catheters could even vary between trials in a single fish (cf. kawakawa no. 14, run numbers 1 and 4, Table 1). Nevertheless, oxygen extraction was usually greater than 50 %. The observation that dye concentrations (% transmittance) did not mirror oxygen extraction changes indicates that the variability was not due to error caused by sampling water outside the exhalant water stream. Variations in oxygen extraction could be due to differences in either water flow over, or blood flow through, various regions of the gill sieve. These variations represent one of the major problems in determining $\dot{V}_G$ from measurements of $\dot{V}_{O_2}$ and oxygen extraction.

The highest $\dot{V}_G$ recorded was $9.01\,l\,min^{-1}\,kg^{-1}$ in a kawakawa (mass=2.03 kg) swimming at $2.22\,L\,s^{-1}$, where L is body length. At low swimming speeds ($<1.9\,L\,s^{-1}$), $\dot{V}_G$ ranged from 1.49 to $4.67\,l\,min^{-1}\,kg^{-1}$, which is somewhat less than has been generally assumed for tuna (Brown and Muir, 1970; Stevens, 1972) but much higher than for most teleosts. $\dot{V}_{O_2}$ at these swimming speeds ranged from 434 to $1485\,ml\,O_2\,kg^{-1}\,h^{-1}$, which is well within the range of values ($3060–5100\,mg\,O_2\,h^{-1}$) recently measured by Graham *et al.* (1989) in large albacore (8–12 kg) swimming at similar speeds in a tunnel respirometer at 13–17 °C. Oxygen consumption measurements of a group of skipjack tuna in a large tank at 24 °C were also similar to those reported here (e.g. Gooding *et al.* 1981; Graham and Laurs, 1982).

Many fish ram ventilate at high swimming speeds, which suggests that the dye-dilution technique could be used for measuring $\dot{V}_G$ in other active fish besides tuna. Also, the highly pulsatile nature of water flow at the anterior end of the buccal cavity of fish breathing normally (Holeton and Jones, 1975; Lauder, 1984) indicates that good dye mixing should be achieved even in resting fish. Consequently, this technique could perhaps be used under both resting and active conditions in fish other than tuna.

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