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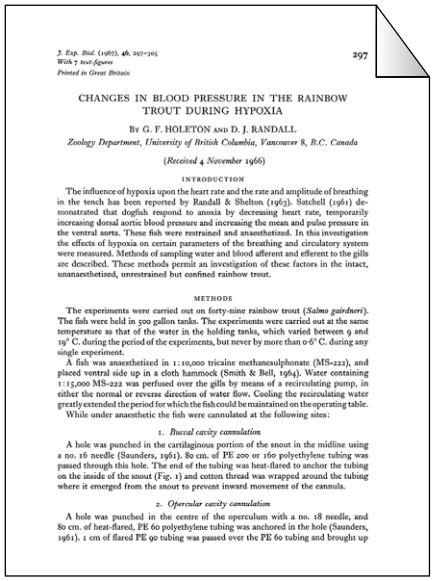
IN VIVO BLOOD AND GUTS PHYSIOLOGY IN FISHES



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CHANGES IN BLOOD PRESSURE IN THE RAINBOW TROUT DURING HYPOXIA

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INTRODUCTION

The influence of hypoxia upon the heart rate and the rate and amplitude of breathing in the tench has been reported by Randall & Shelton (1963). Satchell (1961) demonstrated that dogfish respond to anoxia by decreasing heart rate, temporarily increasing dorsal aortic blood pressure and increasing the mean and pulse pressure in the ventral aorta. These fish were restrained and anaesthetized. In this investigation the effects of hypoxia on certain parameters of the breathing and circulatory system were measured. Methods of sampling water and blood afferent and efferent to the gills are described. These methods permit an investigation of these factors in the intact, unanaesthetized, unrestrained but confined rainbow trout.

METHODS

The experiments were carried out on forty-nine rainbow trout (*Salmo gairdneri*). The fish were held in 500 gallon tanks. The experiments were carried out at the same temperature as that of the water in the holding tanks, which varied between 9 and 19° C. during the period of the experiments, but never by more than 0·6° C. during any single experiment.

A fish was anaesthetized in 1:10,000 tricaine methanesulphonate (MS-222), and placed ventral side up in a cloth hammock (Smith & Bell, 1964). Water containing 1:15,000 MS-222 was perfused over the gills by means of a recirculating pump, in either the normal or reverse direction of water flow. Cooling the recirculating water greatly extended the period for which the fish could be maintained on the operating table. While under anaesthetic the fish were cannulated at the following sites:

1. *Buccal cavity cannulation*

A hole was punched in the cartilaginous portion of the snout in the midline using a no. 16 needle (Saunders, 1961). 80 cm. of PE 200 or 160 polyethylene tubing was passed through this hole. The end of the tubing was heat-flared to anchor the tubing on the inside of the snout (Fig. 1) and cotton thread was wrapped around the tubing where it emerged from the snout to prevent inward movement of the cannula.

2. *Opercular cavity cannulation*

A hole was punched in the centre of the operculum with a no. 18 needle, and 80 cm. of heat-flared, PE 60 polyethylene tubing was anchored in the hole (Saunders, 1961). 1 cm of flared PE 90 tubing was passed over the PE 60 tubing and brought up

Don Stevens writes about the series of five papers from Dave Randall's laboratory published in 1967 on gas exchange in fish. All of the papers are available at http://jeb.biologists.org/content/vol46/issue2.

The series of five papers by Dave Randall and his students at the University of British Columbia (UBC), published over 40 years ago, are remarkable as they were the first mechanistic studies of gas exchange in intact, unanesthetized, unrestrained but confined fish. Since then, each paper has been cited more than 300 times and together they have become classics in the field of fish physiology, paving the way for direct physiological measurements in fish *in vivo*.

Previous studies on respiration in fish had focussed on how much oxygen was removed from the water and how it changed with fish species, environmental conditions (e.g. temperature and salinity) and, especially, how it changed with the animal's level of activity. These studies conducted in the 1940s were the focus of much of the work of Fred Fry in the 1960s at the University of Toronto and by his students, Roly Brett, Bill Beamish, Madan Rao and Narayan Kutty (Fry, 1971). Brett published an intensive and extensive study of the cost of swimming in salmon using a swim tube that he designed specifically for the study (Brett, 1964). In addition, much had been learned about the oxygen binding properties of fish blood *in vitro*, especially through the work of Edgar Black and R. W. Root, and the functional anatomy of fish gills, especially by George Hughes. Thus, in the early 1960s, quite a bit was known about factors affecting gas exchange of fishes and about *in vitro* properties of blood. However, little was known about actual cardiovascular and blood gas parameters in fish while the fish was swimming until Randall published his series of five classic papers in 1967.

Randall, fresh from doing a PhD with Graham Shelton at the University of Southampton, had just come to University of British Columbia (UBC) in Vancouver. His PhD on the control of respiration during hypoxia in fish had led to the question of the functional significance of the decrease in heart rate observed when fish are exposed to hypoxic water. Randall realized that one way to tackle the problem would be to measure gas partial pressures of gases in both media and on both sides of the exchanger – that is, in the blood going into and coming out of the gills and in the water coming into and after passing over the gills. Roly Brett had just published his classic paper on oxygen uptake in salmon based on his work at the Pacific Biological Station at Nanaimo – a short ferry ride away from UBC – and a visit with Roly was very fruitful for two reasons. First, because Brett invited Randall's group, which included myself and George Holeton, to come to his lab at Nanaimo and use his fish swimming tubes. And, second, while there, Randall learned of the methods that Gordon Bell, also at the Nanaimo lab working with Lynwood Smith, had modified to cannulate the dorsal aorta of salmon based on the preliminary efforts of Randall and Brett. Smith and Bell had used their technique to inject radio-opaque dyes into the vasculature of fish and to make preliminary measurements of blood volume in salmonids (Smith and Bell, 1964). Randall was very encouraged by this development; he recognized that it would allow sampling blood after it had passed through the gas exchanger. At the invitation of Brett, Randall joined the Nanaimo group and showed that it was also possible to measure blood pressure in intact, unanesthetized, unrestrained but confined fish (Randall et al., 1965).

Several other factors played important roles for our studies. Ken Wolf had just developed a saline solution suitable for use in salmonids, so-called Courtland saline, which approximated the ionic constituents in trout blood (Wolf, 1963); in the Smith and Bell blood volume study (Smith and Bell, 1964) 1% sodium chloride had been used. Leland Clark had developed the polarographic oxygen electrode in 1956 (Clark, 1956) and John Severinghaus and Freeman Bradley had gone on to develop

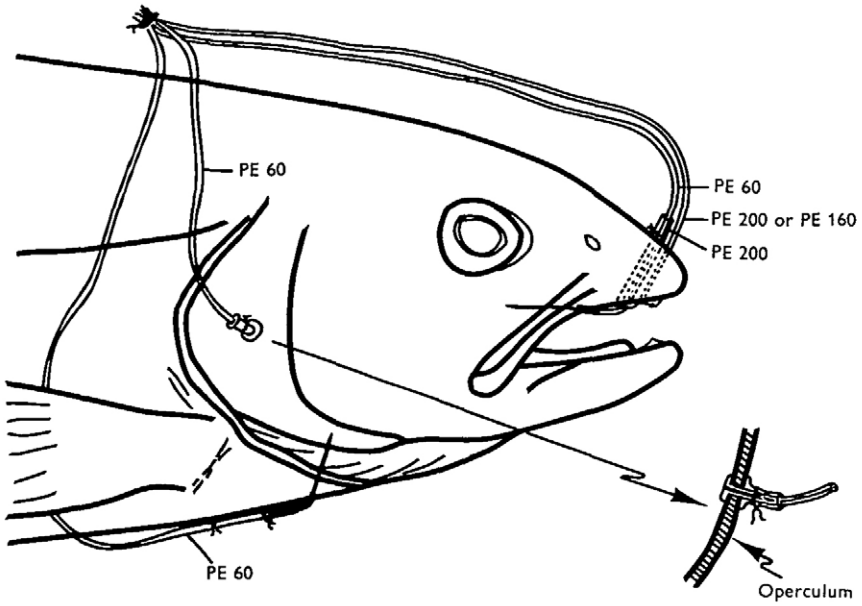

Fig. 1. The position of the cannulae to sample blood and water on either side of the gas exchanger (from Holeton and Randall, 1967a).

small versions suitable for measuring oxygen and carbon dioxide partial pressures and pH in small blood samples (Severinghaus and Bradley, 1958). Just as we were embarking on our studies, commercial versions of these electrodes became available from Beckman (Beckman model 160 physiological blood gas analyser), and the Fisheries Research Board, through Brett, came up with the money to purchase this equipment. Another factor facilitating the research was that in the mid '60s, the national granting agency (called the National Research Council of Canada at that time) had received huge increases in funds that became available for university laboratories so that Randall could fund the work. Thus, Randall had the ideas, the equipment was on the market, and the funds were available to buy the equipment. George Holeton from Calgary and myself from Edgar Black's lab in the medical school at UBC joined Randall's lab in the fall of 1965 and thus we were in the right place at the right time to make the ground-breaking move into physiological measurements in unrestrained, intact unanesthetized fish.

Holeton was going to work on hypoxia and I wanted to continue looking at exercise effects in fish. In an early meeting, Randall explained to us that although we had the method to sample oxygenated blood in the dorsal aorta, we also needed a method to sample venous blood. Holeton went downstairs to the wet lab and returned an hour later with a fish in a bucket with the prototype for the venous sampling cannula and said 'you mean like this?' Although crude by today's standards, a bent syringe needle sutured in place permitted us to measure blood pressure and take samples for blood gas analysis from the ventral aorta. We spent the summer of 1965 working at the biological station in Nanaimo at Brett's invitation using his swim tubes for our experiments and then he allowed us to borrow them so that we could continue our experiments at UBC.

Despite the decades that have elapsed, several aspects of these studies have stood the test of time. For example, the following observations are all still valid: the increases in ventilation and cardiac output during moderate exercise or hypoxia are due more to changes in volume per beat than to changes in respiration rate or heart rate (Holeton and Randall, 1967b; Stevens and Randall, 1967b); moderate exercise or hypoxia results in increases in blood pressure on both sides of the gills (Holeton and Randall, 1967a; Stevens and Randall, 1967a); effectiveness of oxygen uptake by the blood approaches 100%, does not change much during moderate exercise, but is decreased markedly during severe hypoxia (Randall et al., 1967). However, these experiments also had several shortcomings; for example, blood oxygen content was estimated rather than actually measured (Holeton and Randall, 1967b; Stevens and Randall, 1967b) and thus the estimates of cardiac output and ventilation volume were crude estimates rather than exact values. In addition there was wide temperature variation across experimental trials (ranging from 4 to 19°C) because we did not have temperature control of the holding tanks, making some of the comparisons between studies somewhat tenuous. Finally, the hypoxia study was compromised by the fact that CO_2 accumulated as the oxygen was depleted (Holeton and Randall, 1967a; Holeton and Randall, 1967b) and the exercise study was of short duration and not very strenuous (Stevens and Randall, 1967a; Stevens and Randall, 1967b).

The real import of these initial studies was that they resulted in, to use an obnoxious buzzword, a paradigm shift. We had shown to everyone doing experiments in fish physiology that it was possible to make measurements in intact, unanesthetized, unrestrained fish (Fig. 1). It was also clear from our work that measurements taken from restrained fish may be challenged. The shortcomings of the initial studies also provided opportunities for later students (e.g. Kiceniuk and Jones, 1977). Some of the subsequent studies that were stimulated by these initial studies looked at gas exchange in greater detail and actually measured rather than estimated gas content (e.g. Brauner et al., 2000). In addition, it was realized that the method could be applied to the transfer of substances other than gases across fish gills. For example, there are a huge number of studies on ion exchange and acid–base balance, especially by Chris Wood, Steve Perry, Katie Gilmour, Dave Jones, Gord MacDonald, Norbert Heisler, Pat Walsh, Bob Boutilier and their students. Ken Olson and his students have made a career of looking at factors that control peripheral circulation, whereas Tony Farrell and Kurt Gamperl have focussed more on the regulation of the heart and measuring blood flow directly with flow probes under a variety of conditions. Others have used similar techniques to look at turnover of metabolites in fish (e.g. Jim Cameron, Jim Ballantyne, and especially Jean-Michel Weber) or changes associated with stress (George Iwama). Unfortunately, George Holeton was involved in a fatal automobile accident early in his career and so was not able to share in seeing much of the impact his work has had on others.

In summary, this series of papers pioneered the approach that set the stage for an explosion of studies in fish physiology, especially in Canada, that dominated much of comparative physiology for the next three decades. It also is noteworthy that much of the

subsequent work has some connection with UBC – either done by graduate students, postdoctoral students, or visiting scientists in Randall's laboratory.

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