J. exp. Biol. **118**, 173–183 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

THE SEASONAL INTRINSIC CARDIAC PERFORMANCE OF A MARINE TELEOST

BY MARK GRAHAM*

Marine Sciences Research Laboratory, Memorial University of Newfoundland, St. John's, Newfoundland A1C 5S7

AND ANTHONY FARRELL

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S7

Accepted 4 March 1985

SUMMARY

1. An *in situ* heart preparation was used to evaluate cardiac performance in the sea raven, *Hemitripterus americanus*, under physiological inflow and outflow pressure conditions. Winter and summer fish were subjected to an acute 10 °C temperature change from the seasonal ambient value. The maximum cardiac output ($\dot{V}b$) under each temperature condition was determined by altering inflow pressure to the heart.

2. Acute temperature increase produced positive chronotropic and inotropic effects in winter fish. Acute temperature decrease produced a negative chronotropic and inotropic effect in summer fish.

3. The inotropic and chronotropic states of the heart were different in winter and summer fish. Intrinsic heart rate was higher in summer fish at all experimental temperatures. The sensitivity of the summer fish hearts to input pressure was also greater, especially during the warm experimental temperatures.

4. It was evident from heartbeat rate measurements and power output calculations that the advent of summer and winter seasons did not promote any compensatory ability in intrinsic heart function.

INTRODUCTION

Neural and humoral factors have been implicated in inotropic and chronotropic control of cardiac function. Cholinergic input is of prime importance in control of heart rate (f_H) (Gannon & Burnstock, 1969; Laurent, Holmgren & Nilsson, 1983), while adrenergic factors improve the inotropic state of the heart and can exert an excitatory influence on f_H (Gannon & Burnstock, 1969; Randall, 1970; Holmgren, 1977). The relative importance of adrenergic and cholinergic cardiac control is

*Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S7.

Key words: Fish, heart, seasonal.

also affected by the environmental temperature (Priede, 1974; Seibert, 1979; Wood, Pieprzac & Trott, 1979).

In addition to extrinsic factors, there are important intrinsic control mechanisms in the teleost heart (Randall, 1970; Farrell, 1984). For example, the stroke volume (Vs) can undergo marked alteration with small changes in preload pressure (the Starling response) (Johansen, 1962; Randall, 1970; Farrell, MacLeod & Driedzic, 1982; Farrell, MacLeod, Driedzic & Wood, 1983; Stuart, Hedtke & Weber, 1983). Also the heart can intrinsically generate greater output pressures when outflow resistance increases while maintaining cardiac output (homeometric regulation) (Farrell, 1984).

Temperature affects the intrinsic properties of both the heart and tissue metabolism. That is appropriate since $\dot{V}b$ is critical in meeting tissue metabolic needs. While the chronotropic effect of temperature is easily recognized in intact fish, the precise impact on the heart is not readily apparent due to complicating extrinsic factors. Furthermore, as animals acclimate to new temperatures (Prosser, 1958) a number of cell membrane and intracellular adjustments take place. Many of the described changes have been interpreted as adaptations (Hazel & Prosser, 1974). It is likely that cellular alterations occurring in cardiac tissue during the acclimation process affect muscle contractility. The changes, if present, may provide different contractile responses from those seen during acute alterations of temperature.

The present investigation quantifies the direct effects of acute temperature changes on an *in situ* heart preparation. Fish taken during the winter and summer were used to assess any seasonal adjustments of intrinsic heart function, and the maximum (Vs) response to preload was used as an index of contractility.

MATERIALS AND METHODS

Experimental animals and water conditions

Sea ravens, *Hemitripterus americanus* (Gmelin), were caught in Passamoquoddy Bay, and kept in ambient surface sea water at the Huntsman Marine Facility, St Andrews, New Brunswick. The fish were then transported to Mount Allison University and held in sea water (30‰) until experimentation. No food was administered while the fish were in captivity.

Water temperature in the area of capture was 2–3 °C during the winter (January, 1984) and 12–14 °C during the summer (September, 1983) (Fletcher, Kao & Kaya, 1984). During the winter experiments, fish were held in the laboratory at 5 °C for at least 4 weeks, and in the summer at 10 °C for at least 1 week. The summer fish experiments were initiated at a temperature of 13·3 °C with $\dot{V}b$ at approximately 15 ml kg^{-1} min^{-1}. The preload and output pressures of the summer fish were $0{\cdot}09 \pm 0{\cdot}02$ kPa and $3{\cdot}91 \pm 0{\cdot}06$ kPa, respectively. Winter fish experiments were initiated at a temperature of 3·4 °C with $\dot{V}b$ at approximately 9 ml kg^{-1} min^{-1}. The initial $\dot{V}b$ for winter fish was selected based on observations of cardiac output from the previous summer fish experiments after acute

temperature decrease. The preload and output pressures for winter fish were $0{\cdot}12 \pm 0{\cdot}02$ kPa and $4{\cdot}08 \pm 0{\cdot}07$ kPa, respectively.

Experimental protocol

Anaesthetized fish (0·76 mmol l^{-1} ethyl-*m*-aminobenzoate, Sigma) were weighed before surgery. Fish wet weight was used as a reference for setting $\dot{V}b$. Surgery was conducted as described in Farrell *et al.* (1982), the procedure taking 10–20 min. After surgery, the animal was totally transected immediately caudad to the pectoral fins, then placed in a temperature-controlled bath of Cortland's saline. All abdominal viscera were removed.

An initial perfusion of at least 15 min was allowed for tissue flushing (perfusate conditions are outlined in Farrell *et al.* 1982) and heart rate stabilization. After that period, recordings were made on a beat-by-beat basis of $\dot{V}b$, f_H and output pressure. The preload pressure was elevated slowly (<5 mm at each adjustment) until maximum $\dot{V}b$ was attained. When maximum $\dot{V}b$ stabilized (approximately 30 s), another series of recordings was taken before restoring $\dot{V}b$ and preload pressure to original levels. When original $\dot{V}b$ had stabilized, an acute temperature change of about 10 °C was made as follows. In winter fish, the perfusate and water jacket were warmed from $3{\cdot}4 \pm 0{\cdot}2$ °C to $13{\cdot}6 \pm 0{\cdot}3$ °C over $10{\cdot}2 \pm 0{\cdot}8$ min. In summer fish, the perfusate and water jacket were cooled from $13{\cdot}3 \pm 0{\cdot}3$ °C to $4{\cdot}1 \pm 0{\cdot}2$ °C over $19{\cdot}9 \pm 3{\cdot}6$ min. The temperature of the saline in the bath holding the preparation changed much more slowly than that of the perfusate, so the heart tissue temperature was altered primarily by the perfusate passage. The temperature of the myocardium was not measured. We assumed that the heart tissue temperature was stable at or near the perfusate temperature because even though the lagging saline bath solution temperature was still changing, the f_H was stable. Heart rate stability at the altered perfusate temperature was required before the experiments proceeded. When the perfusate temperature change had been completed, and heart rate was stable, the cardiovascular variables were recorded again. Preload was again elevated to achieve maximum $\dot{V}b$ at the new temperature. The experiments were terminated shortly after the recordings made at that stage. Ventricles were excised immediately. After drying with tissue paper, and voiding the contents, wet weight was noted.

Experimental measurements and calculations

The preload and output pressures were measured with pressure transducers (Biotronex Laboratory, Kensington, Maryland). The volume ejected with each heart beat (Vs) was measured with an electromagnetic flow probe (Biotronex Laboratory, BL-5020). The flow probe was calibrated with known volumes of physiological saline at the experimental temperatures. Pressure and flow signals were appropriately amplified (Biotronex Laboratory, BL-630) and displayed on a chart recorder (Biotronex Laboratory, BL-882). Perfusate temperature was measured with a mercury thermometer immediately prior to entering the heart.

Heart rate was determined from pressure traces and $\dot{V}b$ was calculated as:

$$\dot{V}b = Vs \times f_H.$$

The power output of the ventricle was calculated from the product of (output pressure − preload pressure) × $\dot{V}b$, and expressed as $mW\,kg^{-1}$ ventricular wet weight. The resistance of inflow and outflow catheters of the heart was considered in all pressure measurements. All values presented are means ± 1 S.E., except where otherwise stated. Mean values were evaluated for significant differences using Student's *t*-test with $P \leqslant 0{\cdot}05$.

RESULTS

The effects of acute temperature change on f_H are shown in Fig. 1A. After rapid cooling to 4·1 °C the heart rate of summer fish was $34\,min^{-1}$. The winter fish seasonal f_H value (3·4 °C) was $23\,min^{-1}$. Rapid warming of winter fish to 13·6 °C created f_H values that were not significantly different from seasonal summer fish values. The adaptive significance of the apparent asymmetry of sensitivity to acute temperature change by winter and summer animals is considered later. Preload pressure had little or no effect on heart rate (Fig. 2).

Under low preload conditions the power output of summer fish hearts was

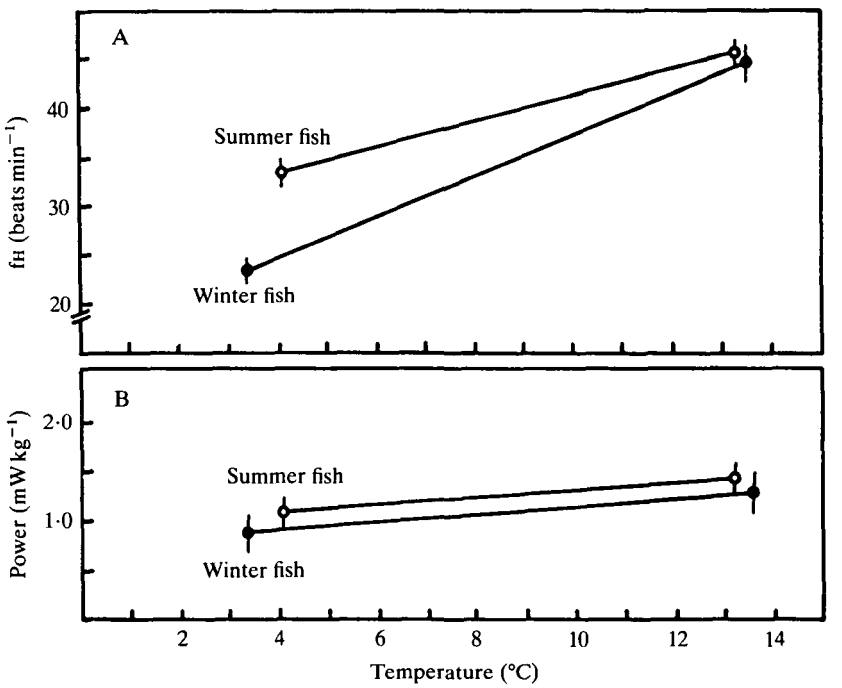

Fig. 1. The effects of seasonal and acute temperature change on intrinsic beat rate of the heart (A) and power production of the ventricle (B). Open symbols are values for summer fish and others are for winter fish. All values are means ± S.E.M. Mean values for cold condition in A were significantly different.

always greater than that of winter fish (Fig. 1B). The power alterations during acute temperature change for the winter and summer fish groups were parallel, and mean values were significantly different only at the lower experimental temperatures. The parallel nature of those values existed even though f_H, a component of $\dot{V}b$, and thus the power equation, had a very different relationship. This pointed to the nature of $\dot{V}b$ changes with temperature (Fig. 2).

$\dot{V}b$ was the product of Vs and f_H. While f_H was positively related to temperature, Vs had an inverse relationship (Fig. 2). Thus under low preload conditions the seasonal winter fish group had the greatest stroke volumes, while the seasonal summer fish group had the lowest. A shift in Vs occurred with acute temperature change, such that a slight average increase occurred when summer fish were cooled and a large decrease occurred when winter fish were warmed. The cooled summer fish results were still significantly different from seasonal winter values, but the acutely warmed winter fish values were not different from the seasonal summer averages. The trends with Vs directly opposed those for f_H, so when summer and winter fish $\dot{V}b$ values were compared against each other under low preload and similar temperature conditions, no significant difference was apparent.

The basic parameter that changed with preload pressure was Vs (Fig. 2). The Vs increased with preload under all seasonal and temperature conditions. The magnitude of the change was greater in summer fish at either experimental temperature. Furthermore, the total Vs change demonstrated required a much smaller change in preload for the summer fish. The $\dot{V}b$ and power data for winter fish at the higher preloads reflected those trends, therefore the values were always significantly lower in that group. The maximum $\dot{V}b$ response to preload was an indicator of heart muscle contractility.

When the ventricle was subjected to a greater end-diastolic volume (as when filling pressure increases), the force of systolic contraction increased, resulting in a greater volume per beat (Starling's law of the heart, see Burton, 1975). In the present study a Starling response was regarded as an increase in Vs per unit inflow pressure per unit ventricular mass (Fig. 3). The Starling response of summer fish was considerably greater than that of winter fish at both experimental temperatures.

DISCUSSION

Heart rate

The suitability of the *in situ* sea raven heart preparation for physiological investigations has been established (Farrell *et al.* 1982, 1983). The control inflow pressures during cold and warm conditions were not significantly different (approx. 1 cmH_2O; 1 kPa = 10·2 cmH_2O) and seem physiological. Chan & Chow (1976) observed sinus venosus pressures of 0·54–2·45 cmH_2O for *Anguilla japonica* at 20 °C. Kiceniuk & Jones (1977) showed a similar range for *Salmo gairdneri* at 10 °C (1·94–2·55 cmH_2O). No *in vivo* studies are known which can validate preload pressure at low temperature.

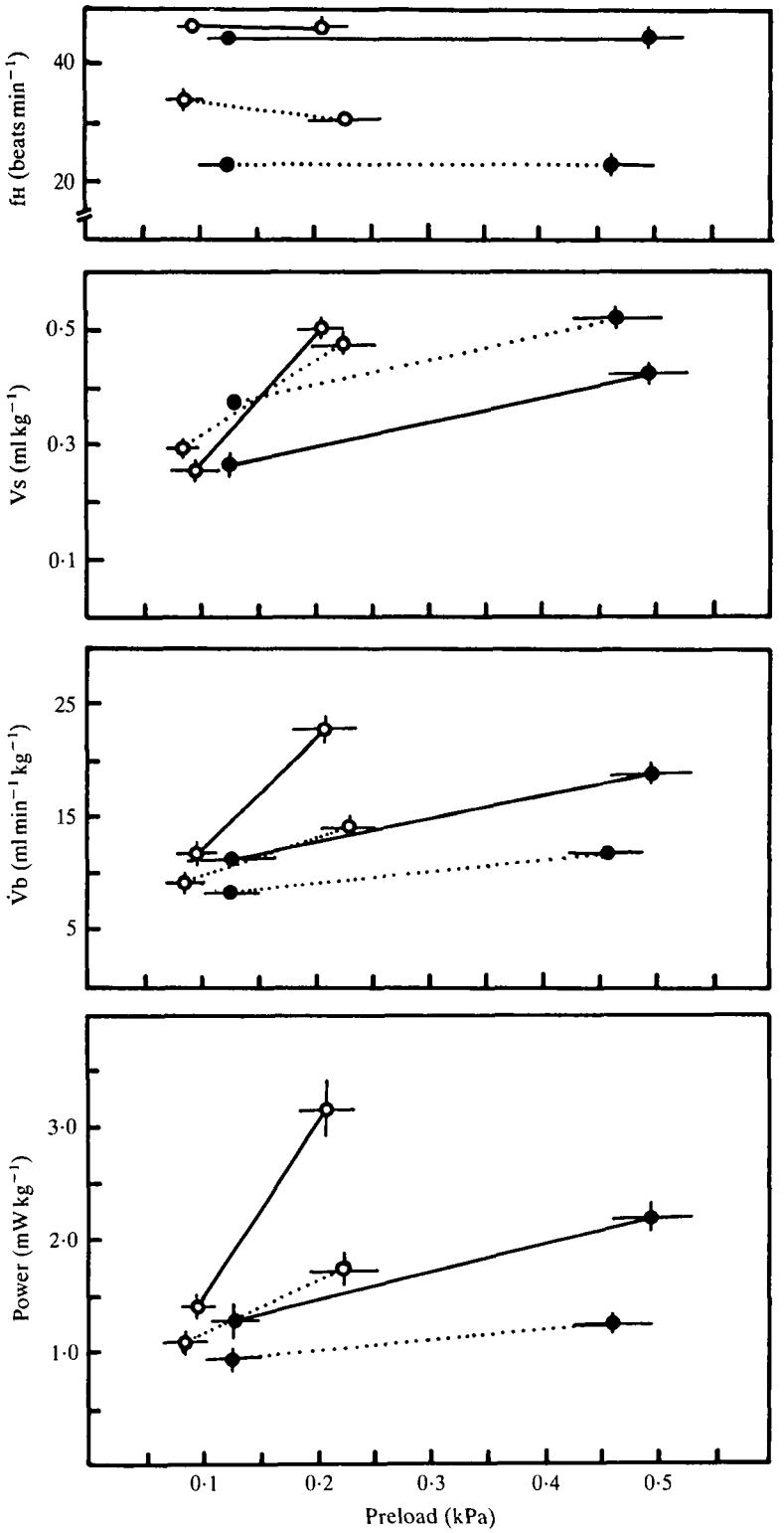
fH (beats min⁻¹)
40
20
Vs (ml kg⁻¹)
0·5
0·3
0·1
V̇b (ml min⁻¹ kg⁻¹)
25
15
5
Power (mW kg⁻¹)
3·0
2·0
1·0
0·1
0·2
0·3
0·4
0·5
Preload (kPa)

Fig. 2

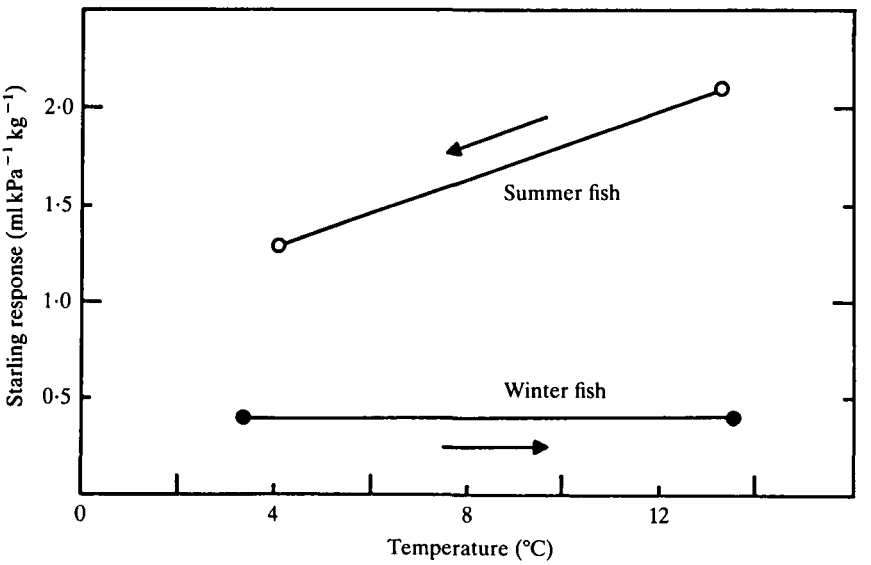

Fig. 3. The Starling response of the sea raven heart in the winter (●) and summer (○) after acute temperature change. The arrows indicate direction of acute temperature change. Input pressure of the heart was at initial or control levels (see Materials and Methods section for exact values).

During the preparation, nerves supplying the heart were severed, thus f_H became solely dependent upon the characteristics of the sino-atrial pacemaker (Farrell *et al.* 1982). Since f_H was very stable under control conditions, any change with acute temperature alteration was interpreted as a variation in the intrinsic rate. The influence of freely circulating endocrine factors was minimized by saline rinsing the vasculature of the heart prior to experimentation: no other circulation reaches the heart. Seasonal differences in any lingering endocrine factors were not determined. The direct role of temperature in setting f_H was only described with certainty during the acute temperature alterations. Seasonal f_H values could be the product of numerous variables which are sensitive to long-term temperature change or other seasonal environmental parameters that ultimately alter the intracellular condition of the heart cells. The effects of seasonal temperature change alone could not be discerned from this study.

The important role played by temperature in setting the intrinsic f_H was well demonstrated. Heart rate was dependent upon temperature during acute heating and cooling (Fig. 1). The temperature dependence varied with the season of study. At warm experimental temperatures the f_H values were not different (Fig. 1). However, when summer fish hearts were rapidly cooled, f_H values stabilized at rates which were significantly greater than those of seasonal winter

Fig. 2. The effects of preload on intrinsic heart beat rate (f_H), stroke volume (V_S), ventricular fluid output ($\dot{V}b$) and ventricular power output for summer (○) and winter (●) fish at cold (...) and warm (——) temperatures. See Materials and Methods section for exact temperature values. All values are means ±S.E.M. (1 kPa = 10·2 cmH_2O).

fish. These observations point to different intrinsic seasonal temperature sensitivities which may have adaptive significance. A probable situation for the sea raven is the case of a summer fish being able to maintain a high f_H when going into colder water.

Precht (1958) described five types of temperature adaptation in poikilothermic animals. By comparing seasonal f_H values with acutely imposed ones, it was possible to categorize the temperature compensation observed here as inverse or type 5. Inverse temperature compensation is a sustained deviation from an original condition during the acclimation period. A change in season from summer to winter (or *vice versa*) brought about no compensatory ability for intrinsic f_H adjustment to temperature. Seibert (1979) with vagotomized European eels, and Harrison (1977), with snail hearts, described partial f_H compensations for temperature change (type 3).

Contractility

Power is a good measure of contractility since it is the product of pressure development and $\dot{V}b$. By altering preload pressure we implimented the Starling response ($\Delta Vs/\Delta$ input pressure/tissue mass) at all experimental temperatures. The difference in the Starling response at various temperatures indicated inotropic states of the myocardium.

The changes in power output and $\dot{V}b$ produced during the Starling response were primarily a result of Vs changes (Fig. 2). Preload had little effect on f_H of the sea raven. These results confirm previous studies with sea raven (Farrell *et al.* 1982, 1983), buffalo sculpin (Stuart *et al.* 1983) and the Atlantic cod (Johansen, 1962), but are contrary to those reported by Randall (1970) for the trout, which demonstrated positive bathmotropic responses (pressure-related chronotropy) to preload.

Inotropic changes also occurred after an acute temperature shift, based on the Starling response (Fig. 3). Those inotropic changes were most apparent in summer fish, which was opposite to the trend seen for changes in intrinsic f_H after acute temperature alteration (Fig. 1A). The maximum contractility of summer fish heart muscle was 5·25 times greater than that of winter fish.

Role of seasonal adaptation in cardiac performance

In some respects, the intrinsic cardiac performance of the sea raven has no compensatory ability for temperature change. That does not mean the fish is poorly adapted, since performance of the heart can be expected to parallel the metabolism of the animal. When inshore water temperature declines, often to near freezing, the sea raven is thought to move into deeper water (to 192 m: Bigelow & Schroeder, 1953), although there is no direct evidence of this. The animal has been known to overwinter in the near-freezing Magdalen shallows of the Gulf of St Lawrence. The lower temperature of the winter habitat would depress metabolic activity. From our casual observation of winter fish, greatly reduced

liver size was apparent, indicating the possibility of limited feeding at that time. The nutritive status of the winter fish is purely speculative without more extensive biological data. However, if extensive feeding limitations are apparent during the winter, production of necessary high energy compounds that fuel heart contraction may be compromised. If feeding, and thus swimming movements are reduced in the colder winter habitat, then seasonal circulatory compensation would not be necessary. Conversely, in the warmer habitat of the summer fish, the heart undergoes positive inotropic and chronotropic alterations, which are effective in producing elevated $\dot{V}b$ even if confronted by intermittent cold stints. It is suggested that the inverse acclimation trends outlined are of no hindrance to the sea raven's normal seasonal lifestyle.

The time that fish were held in the laboratory and consequently denied food, appeared to have no effect on intrinsic heart function. Experimentation took place over a 25-day period during each of the winter and summer periods and no trend of deteriorating power output by the hearts was noticed in either.

An important question is how the heart contends with the increase in blood viscosity as temperature decreases (Graham & Fletcher, 1983, 1984). Cardiac performance, and the influence of perfusate viscosity, can be considered in the power determinations where

$$\text{power} = \dot{V}b \times (\text{outflow pressure} - \text{inflow pressure}).$$

Pressure development = vascular resistance × flow, so the power equation can be rewritten as:

$$\text{power} = \dot{V}b^2 \times Rs,$$

where Rs equals systemic vascular resistance. Cech, Bridges, Rowell & Balzer (1976) showed that winter flounder undergo $\dot{V}b$ and Rs changes of similar magnitude over the 15–5 °C range (Q_{10} about 2·5). If all other factors remain constant, vascular resistance is directly proportional to blood viscosity. Graham & Fletcher (1983) described a blood viscosity Q_{10} of 1·8 over the same temperature range for the winter flounder. It appears that the increase in blood viscosity during seasonal temperature change would not create a problem in power production.

Conclusions

It was clear that the intrinsic ability of summer fish hearts was different from that of winter fish. The summer fish hearts had a much greater sensitivity to acute changes in inflow pressure, and had a larger potential to generate flow. That potential persisted even during the acute cold exposure of summer fish hearts. Change in season did not promote any intrinsic f_H compensation. The differences in seasonal intrinsic cardiac function contributed to the greatly differing maximum cardiac outputs shown for winter and summer fish.

The apparent intrinsic seasonal differences were also evident during acute temperature change experiments. The $\dot{V}b$ of summer fish exposed to an acute temperature gradient was the result of a similar degree of chronotropic and

inotropic involvement. However, the $\dot{V}b$ of winter fish was different in that chronotropic changes with acute temperature variation played the major role.

Although the data suggest no intrinsic cardiac compensatory ability after a seasonal change from summer to winter (or *vice versa*), the observed patterns may have adaptive significance according to the known biology of the animal.

The expert technical assistance of Mr Tom Hart is hereby acknowledged. The arrangement for sea ravens was made possible through Dr J. Foster of the Huntsman Marine Laboratory. The hospitality of Dr J. Bailey during the course of this work was greatly appreciated. Special thanks go to Dr D. Deibel for the generous use of his computer. This research was supported by NSERC, Canada funding to Dr G. Fletcher of the Marine Sciences Research Laboratory, St John's, Newfoundland, and APF. Marine Sciences Research Laboratory Contribution Number 598.

REFERENCES

BIGELOW, H. B. & SCHROEDER, W. C. (1953). *Fishes of the Gulf of Maine*. Fishery bulletin of the fish and wildlife service, Vol. 53, pp. 454–457. Washington: United States Government Printing Office.

BURTON, A. C. (1975). *Physiology and Biophysics of the Circulation*, p. 149. Chicago: Yearbook Medical Publishers, Inc.

CECH, J. J., BRIDGES, D. W., ROWELL, D. M. & BALZAR, P. J. (1976). Cardiovascular responses of the winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Can. J. Zool.* **54**, 1383–1388.

CHAN, D. K. O. & CHOW, P. H. (1976). The effects of acetylcholine, biogenic amines, and other vascular agents on the cardiovascular functions of the eel, *Anguilla japonica*. *J. exp. Zool.* **196**, 13–26.

FARRELL, A. P. (1984). A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. *Can. J. Zool.* **62**, 523–536.

FARRELL, A. P., MACLEOD, K. & DRIEDZIC, W. R. (1982). The effects of preload, afterload, and epinephrine on cardiac performance in the sea raven, *Hemitripterus americanus*. *Can. J. Zool.* **60**, 3165–3171.

FARRELL, A. P., MACLEOD, K., DRIEDZIC, W. R. & WOOD, S. (1983). Cardiac performance during hypercapnic acidosis in the *in situ* perfused fish heart. *J. exp. Biol.* **107**, 415–429.

FLETCHER, G. L., KAO, M. H. & KAYA, K. (1984). Seasonal and phenotypic variations in plasma protein antifreeze levels in a population of marine fish, sea raven (*Hemitripterus americanus*). *Can. J. Fish. aquat. Sci.* **41**, 819–824.

GANNON, B. J. & BURNSTOCK, G. (1969). Excitatory adrenergic innervation of the fish heart. *Comp. Biochem. Physiol.* **29**, 765–773.

GRAHAM, M. S. & FLETCHER, G. L. (1983). Blood and plasma viscosity of winter flounder: influence of temperature, red cell concentration, and shear rate. *Can. J. Zool.* **61**, 2344–2350.

GRAHAM, M. S. & FLETCHER, G. L. (1984). On the low viscosity blood of a cold water, marine sculpin: a comparison with the winter flounder. *J. comp. Physiol. J. Comp. Physiol* **155**B, 455–459.

HARRISON, P. T. C. (1977). Seasonal changes in the heart rate of the freshwater pulmonate *Lymnaea stagnalis* (L.). *Comp. Biochem. Physiol.* **58**A, 37–41.

HAZEL, J. R. & PROSSER, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620–677.

HOLMGREN, S. (1977). Regulation of the heart of a teleost, *Gadus morhua*, by autonomic nerves and circulating catecholamines. *Acta physiol. scand.* **99**, 62–74.

JOHANSEN, K. (1962). Cardiac output and pulsatile aortic flow in the teleost, *Gadus morhua*. *Comp. Biochem. Physiol.* **7**, 169–174.

KICENIUK, J. W. & JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

LAURENT, P., HOLMGREN, S. & NILSSON, S. (1983). Nervous and humoral control of the fish heart: structure and function. *Comp. Biochem. Physiol.* **76**A, 525–542.

PRECHT, H. (1958). Concepts of temperature adaptation of unchanging reaction systems of cold-blooded animals. In *Physiological Adaptation*, (ed. C. L. Prosser), pp. 51–78. Washington, D.C.: American Physiological Society.

PRIEDE, I. G. (1974). The effects of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. exp. Biol.* **60**, 305–319.

PROSSER, C. L. (1958). General summary: the nature of physiological adaptation. In *Physiological Adaptation*, (ed. C. L. Prosser), pp. 167–180. Washington, D.C.: American Physiological Society.

RANDALL, D. J. (1970). The circulatory system. In *Fish Physiology*, Vol. 4, (eds W. S. Hoar & D. J. Randall), pp. 132–172. New York: Academic Press.

SEIBERT, H. (1979). Thermal adaptation of heart rate and its parasympathetic control in the European eel, *Anguilla anguilla* (L.). *Comp. Biochem. Physiol.* **64**C, 275–278.

STUART, R. E., HEDTKE, J. L. & WEBER, L. J. (1983). Physiological and pharmacological investigation of the nonvascularized marine teleost heart with adrenergic and cholinergic agents. *Can. J. Zool.* **61**, 1944–1948.

WOOD, C. M. PIEPRZAK, P. & TROTT, J. N. (1979). The influence of temperature and anemia on the adrenergic and cholinergic mechanisms controlling heart rate in the rainbow trout. *Can. J. Zool.* **59**, 2440–2447.