The Journal of Experimental Biology 208, 1927-1935
Published by The Company of Biologists 2005
doi:10.1242/jeb.01606

Cardiac preload and venous return in swimming sea bass (*Dicentrarchus labrax* L.)

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Accepted 15 March 2005

Summary

Cardiac preload (central venous pressure, P_{CV}), mean circulatory filling pressure (MCFP), dorsal aortic blood pressure (P_{DA}) and relative cardiac output ($\dot{Q}$) were measured in sea bass (*Dicentrarchus labrax*) at rest and while swimming at 1 and 2 *BL* s^{-1}. MCFP, an index of venous capacitance and the upstream venous pressure driving the return of venous blood to the heart, was measured as the plateau in P_{CV} during ventral aortic occlusion. Compared with resting values, swimming at 1 and 2 *BL* s^{-1} increased $\dot{Q}$ (by 15±1.5 and 38±6.5%, respectively), P_{CV} (from 0.11±0.01 kPa to 0.12±0.01 and 0.16±0.02 kPa, respectively), MCFP (from 0.27±0.02 kPa to 0.31±0.02 and 0.40±0.04 kPa, respectively) and the calculated pressure gradient for venous return (ΔP_V, from 0.16±0.01 kPa to 0.18±0.02 and 0.24±0.02 kPa, respectively), but not P_{DA}. In spite of an increased preload, the increase in $\dot{Q}$ was exclusively mediated by an increased heart rate (f_H, from 80±4 beats min^{-1} to 88±4 and 103±3 beats min^{-1}, respectively), and stroke volume (V_s) remained unchanged. Prazosin treatment (1 mg kg^{-1} M_b) abolished pressure and flow changes during swimming at 1 *BL* s^{-1}, but not 2 *BL* s^{-1}, indicating that other control systems besides an α-adrenoceptor control are involved. This study is the first to address the control of venous capacitance in swimming fish. It questions the generality that increased $\dot{Q}$ during swimming is regulated primarily through V_s and shows that an increased cardiac filling pressure does not necessarily lead to an increased V_s in fish, but may instead compensate for a reduced cardiac filling time.

Key words: cardiac preload, cardiac output, exercise, heart rate, mean circulatory filling pressure, prazosin, sea bass, stroke volume, teleost, venous capacitance, venous return.

Introduction

Although it is well known that fish increase cardiac output ($\dot{Q}$) during exercise to meet increased metabolic demands, there is a controversy concerning the basic hemodynamic mechanisms underlying this increase. One view is that stroke volume (V_s) and, to a lesser extent, heart rate (f_H) are responsible for the rise in $\dot{Q}$ in swimming fish, especially in salmonids (Dunmall and Schreer, 2003; Farrell, 1991; Farrell and Jones, 1992; Jones and Randall, 1978; Kiceniuk and Jones, 1977). This view has been questioned by others, who suggest that increased f_H is the primary means of increasing $\dot{Q}$ during swimming (Altimiras and Larsen, 2000; Axelsson et al., 1994; Cooke et al., 2003; Korsmeyer et al., 1997; Lefrancois et al., 1998; Priede, 1974). While this difference in opinion may exist because different species do indeed have different strategies for increasing $\dot{Q}$, we know little of one of the primary determinants of V_s in fish, i.e., cardiac preload (= central venous pressure, P_{CV}). Ventricular end-systolic volume, at least in rainbow trout, is close to zero and hence cannot be reduced much further during exercise (Forster and Farrell, 1994; Franklin and Davie, 1992). Thus, any increase in V_s has to come about by increasing end-diastolic volume, which must come about through increased P_{CV} and a concurrent increase in the myocardial force of contraction due to the Frank-Starling relationship (Farrell, 1991; Farrell and Jones, 1992). Consequently, without information on the changes in P_{CV} during swimming it is difficult to evaluate fully the role of increasing V_s.

This line of logic ignores the fact that under steady-state conditions, $\dot{Q}$ equals venous return and the heart can pump only what it gets back from the venous circulation. Moreover, any increase in P_{CV} during exercise will reduce the pressure gradient for venous return (flow) to the heart from the venous periphery, if end-capillary blood pressure and venous resistance remain unaltered. Thus, in addition to increasing cardiac filling pressure, a proportional increase in peripheral venous pressure is expected to ensure that venous return can

match the increase in $\dot{Q}$. Venous return can be estimated from measurements of P_{CV} and venular blood pressures.

The mean circulatory filling pressure (MCFP) is an index of venous capacitance. It is measured as the venous pressure after a short (5–10 s) cardiac arrest and also represents the upstream venous (venular) pressure that drives venous return (Pang, 2001; Rothe, 1986, 1993; Sandblom and Axelsson, 2005). MCFP can increase due to an increased smooth muscle tone and/or a decreased compliance in venous capacitance vessels (Conklin et al., 1997; Hoagland et al., 2000; Olson et al., 1997; Pang, 2001; Rothe, 1986, 1993; Zhang et al., 1998). While comprehensive studies have examined the nervous and humoral control of venous capacitance in unaesthetized fish under resting conditions (Conklin et al., 1997; Hoagland et al., 2000; Olson et al., 1997; Sandblom and Axelsson, 2005; Zhang et al., 1998), none have considered the changes in venous capacitance that are likely to occur during exercise. Furthermore, basic information on P_{CV} during exercise is limited to a few studies and what data exist are compromised by noisy signals and/or experiments on a small number of animals (Jones and Randall, 1978; Kiceniuk and Jones, 1977; Stevens and Randall, 1967).

The primary objective of this study was therefore to measure changes in P_{CV} and MCFP in a fast swimming teleost, the European sea bass (*Dicentrarchus labrax* L.). By combining P_{CV} and MCFP measurements, it was also possible to assess the degree to which the pressure gradient for venous return, venous capacitance and cardiac preload change during the periods of increased $\dot{Q}$ associated with exercise. Also, the role of α-adrenoceptor control of these responses was examined, since Zhang et al. (1998) identified that venous capacitance in resting rainbow trout (*Oncorhynchus mykiss*) can be altered by α-adrenergic mechanisms.

Materials and methods

Animals

Results from 13 sea bass (279–648 g and 31–38 cm) are presented in this study. The fish were obtained from the Ferme Marine des Baleines (Ile de Ré, France) and maintained at CREMA under a natural photoperiod in indoor 400 l fibre glass tanks supplied with recirculating, biofiltered seawater at ambient temperature (21–24°C). The fish were fed commercial dry pellets on a regular basis.

Surgical procedures

Prior to surgery the fish were anaesthetized in seawater containing MS-222 (approx 100 mg l^{-1}) and placed on water-soaked foam on a surgery table. During surgery, the fish was covered with wet tissue paper and the gills were continuously irrigated with aerated, chilled (~11–16°C) seawater containing MS-222 (~50 mg l^{-1}). The ventral aorta was exposed with an incision on the right side of the isthmus and dissected free. A Perspex cuff-type 20 MHz Doppler flow probe (Iowa Doppler products; Iowa City, IA, USA), with an inner diameter of 1.8–2.0 mm, was positioned around the aorta proximal to the bulbus to measure relative changes in $\dot{Q}$ (Fig. 1). Also, a cuff-type vascular occluder (i.d. 2.5–4.3 mm) was positioned posterior to the flow probe to obtain zero-flow during measurement of MCFP. The occluder was constructed from Perspex, a heat-flared water-filled PE-50 catheter and a piece of latex rubber (model Thin, Dental Dam, Coltène/Whaledent Inc, USA and Canada). The rubber was tied with a 4–0 suture around the flared end of the PE tubing (Fig. 1). The sinus venosus was non-occlusively cannulated to measure P_{CV} (Altimiras and Axelsson, 2004). Briefly, the operculum was retracted and the left lateral part of the ductus of Cuvier was carefully exposed and dissected free with an incision between the cleithrum and the fifth branchial arch. A small portion of the vessel wall was lifted with forceps and secured with a 4–0 suture, allowing the vessel to be gently lifted during the cannulation procedure. This procedure prevents blood loss, and is an improvement on the method used by Farrell and Clutterham (2003). A PE-50 catheter, with 2–3 side-holes to keep it patent and a bubble 1 cm from the tip, was inserted into the ductus of Cuvier through a small incision made close to the suture holding the vessel. A 4–0 suture was used to close the vessel wall around the catheter, leaving the bubble located on the luminal side of the vessel wall. The catheter was secured with silk sutures to the skin. The third efferent branchial artery on the left side was occlusively cannulated to measure dorsal aortic pressure (P_{DA}). To do this, the first two gill arches were retracted to expose the third gill arch, which was gently

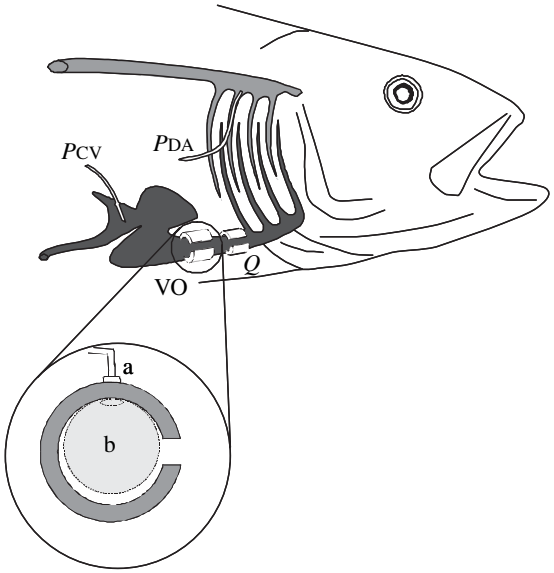

Fig. 1. Arrangement of the Doppler flow probe ($\dot{Q}$) and vascular occluder (VO) on the ventral aorta, and catheters for blood pressure measurements in sinus venosus (P_{CV}) and the efferent branchial artery (P_{DA}). Close to the slit in the cuff of the occluder a hole was drilled using a 20-gauge injection needle. The luminal side of the hole was countersunk using a dental drill. The catheter (a) was pulled from the luminal side with the rubber facing the lumen. Inflation of the rubber thus occluded the aorta located inside the cuff (b). This VO is similar to the one used on trout by Zhang et al. (1998).

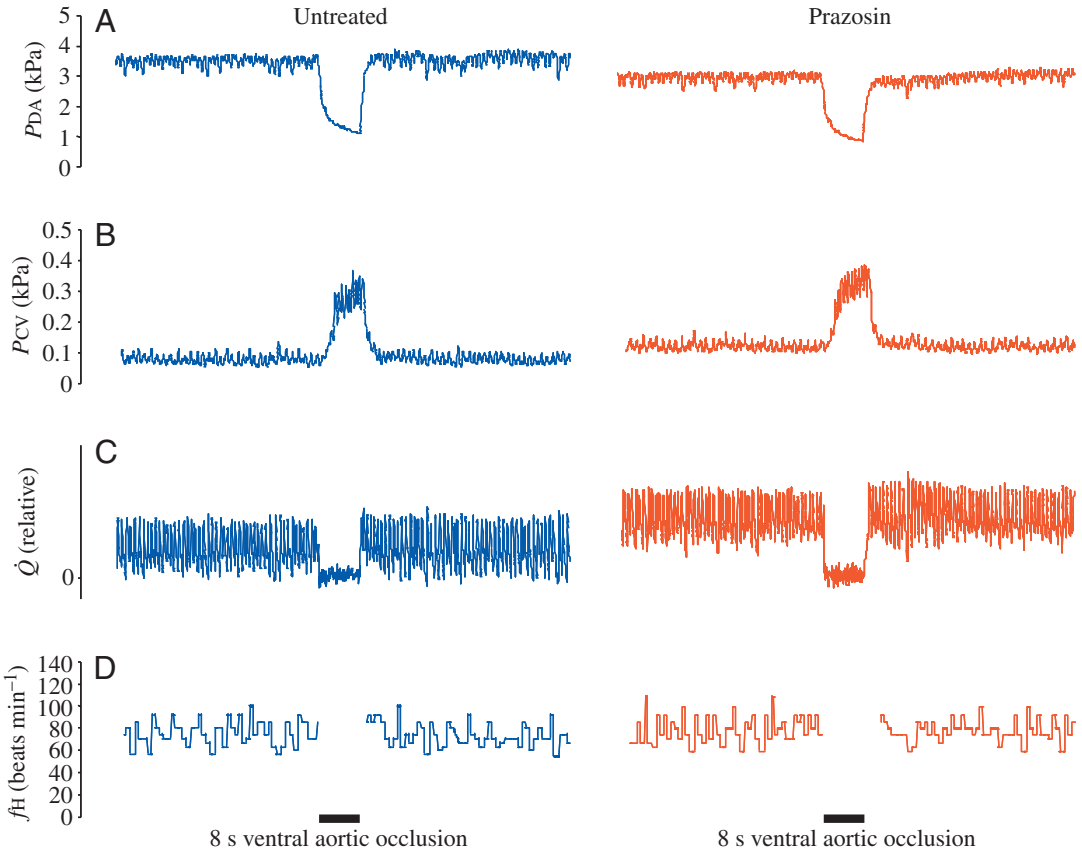

Fig. 2. Representative original recordings during ventral aortic occlusion in a resting 530 g sea bass (*Dicentrarchus labrax*). Before (left-hand panel) and 1.5–2 h after (right-hand panels) prazosin treatment (1 mg kg^{-1} M_b). (A) Dorsal aortic pressure (P_{DA}); (B) central venous pressure (P_{CV}); (C) relative cardiac output in kHz ($\dot{Q}$); and (D) heart rate (f_H). Mean circulatory filling pressure (MCFP) was calculated from the average venous plateau pressure between the 5th and 7th seconds during the ventral aortic occlusion.

retracted. The branchial artery was dissected free close to the angle of the branchial arch, occluded upstream with a 4–0 silk suture and cannulated downstream with a tapered PE-50 catheter (Fig. 1). The catheter was secured with 3–0 silk sutures around the gill arch and secured to the skin with 3–0 silk sutures. The catheters and the lead from the flow probe were collectively secured with a 3–0 suture to the back of the fish. Both blood pressure catheters were filled with physiological saline (0.9% NaCl). Following surgery, the fish were revived in fresh seawater, placed in plastic floating tubes in the holding tanks and allowed a 24 h recovery before experiments commenced.

Experimental protocol

A stainless steel, Brett-type swimtunnel respirometer was used in the present study. This tunnel had been designed to exercise individual fish in a non-turbulent water flow with a uniform cross-sectional water velocity. The total water volume was 48 l and the swim chamber had a square cross-sectional area of 290 cm^2. A propeller downstream of the swim chamber generated water flow. The flow in the swimtunnel was calibrated (Marsh-McBirney 200 flow meter; Frederick, MD, USA) in cm s^{-1}, which was converted to swimming speeds in body lengths s^{-1} (*BL* s^{-1}). The respirometer was thermostatted by immersion in a large outer stainless steel tank that received a flow of aerated water. Since venous pressure is low readings can be affected by any changes in the pressure head on the propeller in the swim-channel when water velocity is changed. To minimise this problem the lid to the swim chamber was removed. Water pressure was measured with a saline-filled catheter immersed in the channel, and no pressure fluctuations were observed at the swimming speeds used (up to 2 *BL* s^{-1}). The swim channel was covered with an opaque black plastic sheet to avoid visual disturbance of the fish.

All experiments were conducted at a temperature of 22°C. The experimental protocol started with a 2 min recording at rest, i.e., the fish oriented into a low water velocity and maintaining position without swimming. MCFP was measured at rest by occluding the ventral aorta for 8 s. Water velocity was then gradually increased over a 5 min period until the fish reached a swimming speed of 1 *BL* s^{-1}, which was maintained for 15 min. MCFP was remeasured at the end of this period. The same procedure was used for a swimming speed of 2 *BL* s^{-1}. Water velocity was then reduced to the resting condition over a 2 min period and an α-adrenoceptor antagonist (prazosin, 1 mg kg^{-1} M_b; Pfizer, Sandwich, UK) was administered *via* the venous catheter. The entire protocol was repeated 1.5–2.0 h later. Preliminary experiments had revealed that an exercise period of 15 min was well beyond the time necessary to establish stable cardiovascular measurements in untreated fish.

Successful ventral aortic occlusion always resulted in a rapid fall in P_{DA}, a rise in P_{CV} and a complete cessation of ventral aortic flow (Fig. 2). Although the ventral aorta was easily accessible, the vessel proved to be relatively fragile as compared with rainbow trout and several sea bass died due to

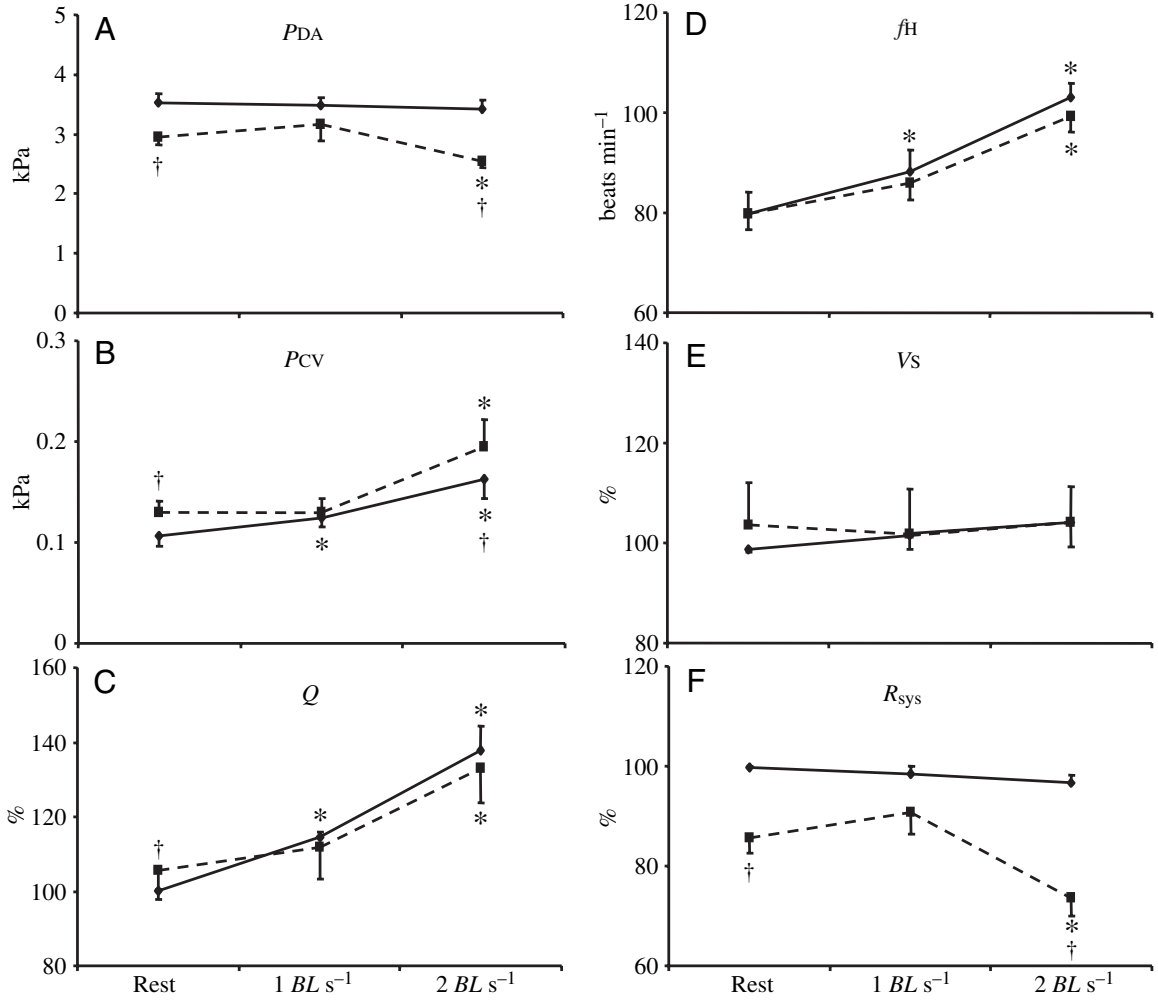

Fig. 3. Mean cardiovascular variables at rest and while swimming at 1 and 2 BL s^{-1} in the sea bass, *Dicentrarchus labrax*. Solid lines represent untreated fish (N=8–9) and hatched lines (N=7) represent fish after treatment with prazosin (1 mg kg^{-1} M_b). The measured and calculated variables are (A) dorsal aortic pressure (P_{DA}); (B) central venous pressure (P_{CV}); (C) cardiac output ($\dot{Q}$); (D) heart rate (f_H); (E) stroke volume (V_s) and (F) systemic resistance (R_{sys}) All values are means ± S.E.M. *Statistically significant difference ($P \leqslant 0.05$) from the resting values within a treatment group, and †statistically significant difference ($P \leqslant 0.05$) between the values before and after prazosin treatment.

fatal rupture of the ventral aorta during occlusion. This generally occurred after the first occlusion and might have been due to mechanical abrasion from the vascular occluder during recovery. Another unusual finding was the low occurrence of blood clotting in the catheters during routine surgery and this was the reason why heparin was omitted from the saline.

Data acquisition, calculations and statistical analysis

Both blood pressure catheters were connected to pressure transducers (model DPT-6100, pvb Medizintechnik, Kirchseeon, Germany), calibrated against a static water column with the water surface of the swim channel serving as the zero reference pressure. The signals from the pressure transducers were amplified with a 4ChAmp amplifier (Somedic, Hörby, Sweden). Relative cardiac output ($\dot{Q}$) was recorded with a directional-pulsed Doppler flow meter (model 545C-4, University of Iowa, USA). The digital signals were fed into a portable computer running a custom made program, General Acquisition (Labview version 6.01, National Instruments, Austin, TX, USA).

Heart rate (f_H) was obtained from either pulsatile pressure or flow records. Relative changes in cardiac stroke volume (V_s) were calculated as $\dot{Q}/f_H$. Total systemic resistance (R_{sys}) was calculated from the pressure drop in the circulation divided by cardiac output $R_{sys}=(P_{DA}-P_{CV})/\dot{Q}$. For the V_s, $\dot{Q}$ and R_{sys} calculations raw data was used and the initial untreated resting value for each fish was arbitrarily set to 100%. The plateau pressure in P_{CV} during ventral aortic occlusion was assumed to equal mean circulatory filling pressure (MCFP), and the average pressure between the 5th and 7th seconds of ventral aortic occlusion is reported here. This time interval is sufficient to obtain reasonably steady plateau values, while minimising the compromising effects of barostatic reflexes (Sandblom and Axelsson, 2005). A similar method has been used previously to measure MCFP in the trout (Hoagland et al., 2000; Zhang et al., 1998). No correction for inequalities in blood remaining in the

Table 1. *Changes in cardiac output, heart rate and stroke volume from nine individual sea bass,* Dicentrarchus labrax*, at rest and while swimming at 1 and 2* BL s^{-1}

		$\dot{Q}$ (%)			f_H (beats min^{-1})			V_S (%)		
	Fish	Rest	1 *BL* s^{-1}	2 *BL* s^{-1}	Rest	1 *BL* s^{-1}	2 *BL* s^{-1}	Rest	1 *BL* s^{-1}	2 *BL* s^{-1}
Untreated	1	101	119	174	73	91	111	97	92	110
	2				93	96	117			
	3	100	108	143	55	58	93	100	104	85
	4	98	119	136	80	88	94	95	104	112
	5	100	113	146	78	88	106	100	100	107
	6	100	109	108	85	97	108	101	95	84
	7	103	117	132	71	85	92	99	94	98
	8	100	115	131	93	102	109	100	106	113
	9	98	116	132	89	90	97	99	117	123
Prazosin	1	118	122	165	79	92	103	105	93	114
	2									
	3	121	135	153	62	70	90	108	107	94
	4	109	119	143	82	86	89	103	107	124
	5	110	119	134	84	91	99	102	102	105
	6	59	64	89	84	95	111	58	56	67
	7									
	8	115	111	121	83	79	109	130	132	105
	9	109	113	124	83	88	94	119	116	119

arterial circulation after occlusion was performed, because the difference was assumed to be negligible due to the large differences in compliances and volumes of the arterial and the venous compartments (Rothe, 1993; Zhang et al., 1998). The pressure gradient (ΔP_V) for venous return was calculated as

$$\Delta P_V = \text{MCFP} - P_{CV} . \quad (1)$$

Resistance to venous return (R_v) was calculated as

$$R_v = (\text{MCFP} - P_{CV})/\dot{Q} , \quad (2)$$

assuming that $\dot{Q}$ equals venous return.

Data (mean values ±S.E.M.) are presented only for fish that performed steady state swimming at both water velocities. Partial data from fish that exhibited either erratic swimming behaviours, burst swimming, died after prazosin treatment, lost a catheter, or the ventral aorta ruptured were discarded. Cardiovascular data were taken from the last 90 s of each exercise period. For the calculations of venous variables, i.e. Fig. 4, P_{CV} was taken as the average of a 30 s period before ventral aortic occlusion. Wilcoxon matched pairs signed-ranks test, with a fiduciary level of $P \leqslant 0.05$ was used to evaluate statistically significant differences in cardiovascular variables at different swimming speeds and between treatments. To compensate for multiple two-group comparisons, a modified Bonferroni-test was applied (Holm, 1979).

Results

Cardiovascular responses to swimming in untreated fish

Fig. 2 shows representative resting cardiovascular recordings for an individual fish during ventral aortic occlusion. Fig. 3 summarises the mean data for fish at rest and during steady state swimming at 1 and 2 *BL* s^{-1}, while Table 1 shows changes in $\dot{Q}$, V_s and f_H for individual fish. Exercise increased $\dot{Q}$ by 15±1.5% and 38±6.5% of the resting value, respectively, at 1 and 2 *BL* s^{-1}. These increases in $\dot{Q}$ were mediated by significant increases in f_H from 80±4 beats min^{-1} at rest to 88±4 beats min^{-1} at 1 *BL* s^{-1} and 103±3 beats min^{-1} at 2 *BL* s^{-1}. By contrast, V_s remained unaltered during exercise. Nevertheless, the increases in $\dot{Q}$ were associated with significant rises in P_{CV} from 0.11±0.01 kPa at rest to 0.12±0.01 kPa at 1 *BL* s^{-1} and 0.16±0.02 kPa at 2 *BL* s^{-1}. However, P_{DA} and R_{sys} were unchanged during exercise.

Fig. 4 shows the rise in P_{CV} during exercise and illustrates changes in venous variables during exercise. In addition to an increase in P_{CV} with increased swimming speed, MCFP increased significantly (0.27±0.02 kPa at rest to 0.31±0.02 kPa and 0.40±0.04 kPa, respectively). Because the rise in MCFP was proportionally larger than that in P_{CV}, ΔP_V increased significantly from 0.16±0.01 kPa at rest to 0.18±0.02 kPa at 1 *BL* s^{-1} and 0.24±0.02 kPa at 2 *BL* s^{-1}, whereas R_v remained unchanged.

Effects of α-adrenoceptor blockade on cardiovascular performance during swimming

Blockade of α-adrenergic receptors with prazosin showed that the cardiovascular system is at least partially controlled *via* α-adrenoceptors, both at rest as well as during exercise (Figs 3 and 4). At rest, prazosin treatment significantly decreased R_{sys}, producing a significant arterial hypotension while P_{CV} increased significantly.

During swimming after prazosin treatment, the increases in P_{CV} and f_H were absent at 1 *BL* s^{-1}, but not at 2 *BL* s^{-1}. In fact, the increase in P_{CV} was accentuated significantly at 2 *BL* s^{-1}

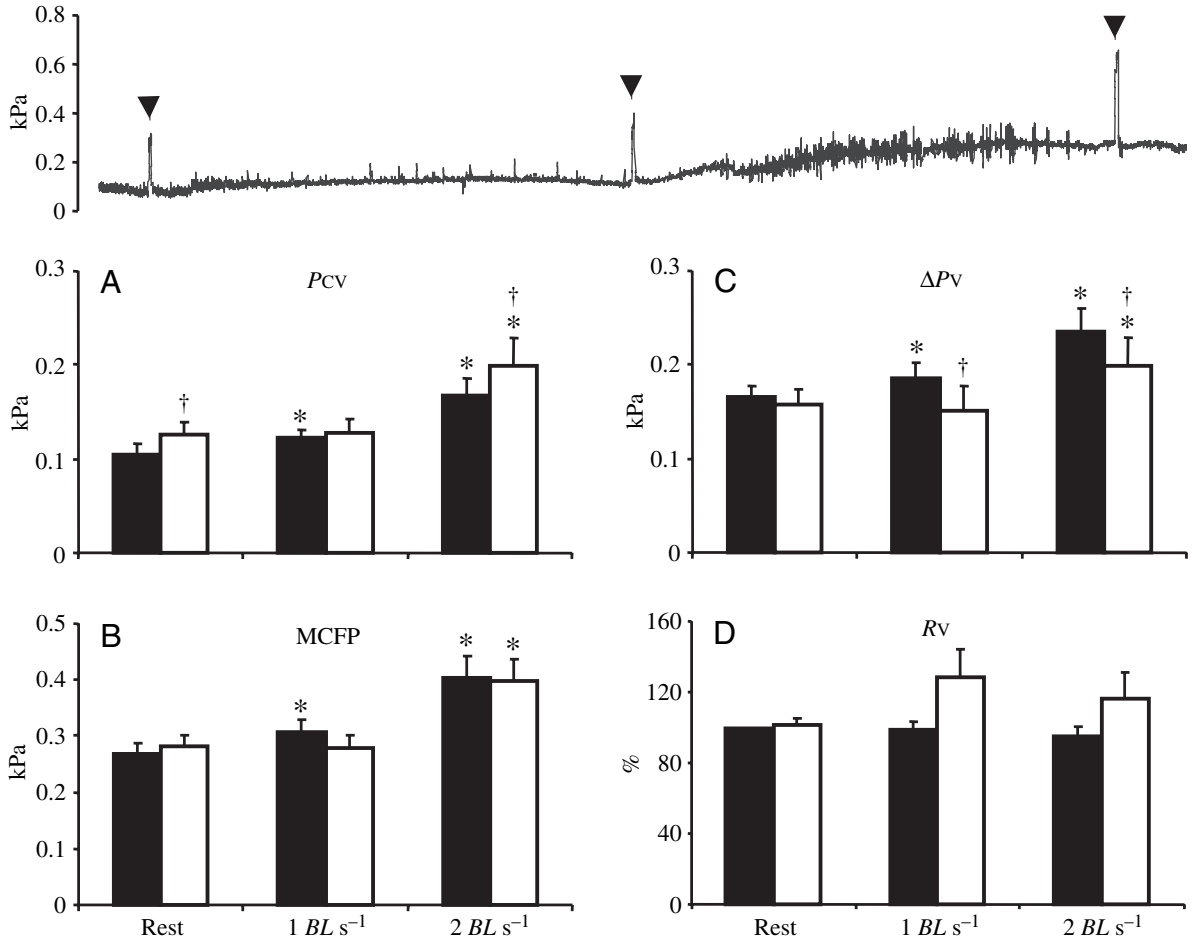

Fig. 4. Upper trace shows a representative recording of venous pressure during a full swim protocol (~30 min) in an untreated sea bass, *Dicentrarchus labrax*. Ventral aortic occlusions for MCFP measurements at the end of each swim speed (rest, 1 *BL* s^{-1} and 2 *BL* s^{-1}, respectively) are marked with vertical arrows. Lower panels (A–D) show mean values (+ S.E.M.) of measured and calculated venous variables at rest and while swimming at 1 and 2 *BL* s^{-1}. Filled bars represent untreated fish (N=8–9) and open bars (N=7) represent fish after treatment with prazosin (1 mg kg^{-1} M_b). The variables are *A*, central venous pressure (P_{CV}); *B*, mean circulatory filling pressure (MCFP); *C*, pressure gradient for venous return (ΔP_V) and *D*, resistance to venous return (R_v). *Statistically significant difference ($P \leq 0.05$) from resting values within each treatment and statistically significant difference ($P \leq 0.05$) between values before and after prazosin treatment. For a detailed description on calculations of mean values, see Materials and methods section.

after prazosin. Prazosin also significantly accentuated the decrease in R_{sys} at 2 *BL* s^{-1} (from the resting value of 85.7±3.2 to 73.5±3.6%) and the corresponding hypotension (P_{DA} from 2.9±0.1 to 2.5±0.1 kPa). Again, V_s remained unchanged throughout the swim challenges after prazosin treatment despite the increase in P_{CV} (Fig. 3). After prazosin, MCFP also remained unaltered at 1 *BL* s^{-1}, but increased significantly from 0.28±0.02 kPa at rest to 0.40±0.05 kPa during swimming at 2 *BL* s^{-1}, but no more so than before prazosin treatment (Fig. 4). As a result ΔP_V increased significantly compared with the resting value at 2 *BL* s^{-1}, although this response was not as pronounced as pre-prazosin treatment. R_v remained unaltered throughout the experiment (Fig. 4).

Discussion

In its natural pelagic habitat, sea bass are subjected to strong water currents requiring long periods of sustained exercise. This ecological fact makes sea bass an excellent experimental animal for exercise studies. However, existing cardiovascular data of sea bass is sparse. Axelsson et al. (2002) reported a resting f_H of 51 beats min^{-1} at 16°C. In the present study at 22°C, f_H was 80 beats min^{-1}, a difference that would represent a Q_{10} of 2.1 between the two studies and suggests that the difference was simply a temperature effect. Furthermore, judging from the variable heart rate in resting fish (Fig. 2), it is likely that the fish had a functional cholinergic tone, which is indicative of an acceptable decay in post-surgical stress (Campbell et al., 2004).

Hemodynamics of venous return and cardiac filling pressure in sea bass

The present study is the first to demonstrate an active control of the venous vasculature during exercise in any species of fish.

Very few studies have successfully measured cardiac filling pressure during swimming in teleost fish. Kiceniuk and Jones (1977) found that P_{CV} in the common cardinal vein of four rainbow trout increased significantly during swimming only when the fish swam at critical swimming speed (U_{crit}), and not at intermediate swimming speeds. This finding is surprising because V_s increased significantly at intermediate swimming speeds, suggesting that these increases in V_s were not a result of increased filling pressure. It is likely that increased adrenergic stimulation of the heart, which is known to both increase during swimming (Axelsson, 1988) and increase the sensitivity of the heart to filling pressure (Farrell et al., 1986) permitted these increases in V_s without a concomittent increase in P_{CV}. Stevens and Randall (1967) measured venous pressure and flow in the subintestinal vein (= hepatic portal vein), and found that venous pressure increased whereas flow decreased. Since the hepatic portal vein drains the gastrointestinal tract and is located upstream of the liver, and arterial gut blood flow decreases during exercise (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Farrell et al., 2001; Thorarensen et al., 1993), it is uncertain to what extent these changes directly affected cardiac performance (for further discussion, see Jones and Randall, 1978).

In the present study, $\dot{Q}$ and f_H increased during swimming. The increase in f_H would have reduced cardiac filling time, but it is clear that the observed increase in preload would have compensated for this, leaving V_s unchanged. As pointed out in the introduction, the increase in P_{CV} in itself could decrease the pressure gradient driving venous return from the periphery to the heart. However, a proportionally larger increase in MCFP ensured that the pressure gradient for venous return actually increased and since R_v was unchanged, venous return would be increased to support the increase in $\dot{Q}$ (Fig. 4).

The increase in MCFP could be attributed to either an increased venous tone, a decreased venous compliance or a combination of both (Conklin et al., 1997; Hoagland et al., 2000; Olson et al., 1997; Pang, 2001; Rothe, 1986, 1993; Zhang et al., 1998). In rainbow trout, adrenaline increases venous tone through an α-adrenergic control and decreases venous compliance (Sandblom and Axelsson, 2005; Zhang et al., 1998). Since vascular capacitance curves could not be constructed in the present study, we do not know the exact mechanism for the increase in MCFP. Nevertheless, the observation that the increases in MCFP, P_{CV}, $\dot{Q}$, ΔP_V and f_H during swimming at $1\ BL\ s^{-1}$ were abolished after α-adrenoceptor blockade (Figs 3 and 4), suggests an important α-adrenergic control mechanism for the venous vasculature in sea bass during exercise, which can mobilize venous blood towards the heart and increase cardiac preload. This control mechanism was evident in resting fish as well. About 2 h after prazosin treatment, resting cardiovascular variables in the sea bass were significantly altered (Figs 3 and 4); P_{CV} increased and both R_{sys} and P_{DA} decreased. It is unlikely that this increase in P_{CV} was mediated by either an increased transcapillary fluid uptake, thus increasing blood volume, or an up-regulation of some compensatory vasoactive system since this would have affected MCFP as well. The importance of an α-adrenergic tone on the arterial side of the circulation has been previously demonstated at rest and during swimming in other fish species (Axelsson and Fritsche, 1991; Axelsson and Nilsson, 1986; Smith, 1978), and was confirmed here because after prazosin treatment sea bass could not maintain R_{sys} at $2\ BL\ s^{-1}$ and suffered a major systemic hypotension. In view of this, it could be argued that the increase in P_{CV} was only a consequence of the reduction in R_{sys}, but then MCFP would not have increased. Instead, the accentuated reduction in arterial pressure at $2\ BL\ s^{-1}$ possibly triggered the activation of some unknown vasoactive system, acting primarily on the venous vasculature. One concern with the present study is that an increased adrenergic tone on resistance vessels may have counteracted a further decrease in R_{sys} during exercise and resulted in an unaltered resistance in untreated fish. It is unknown whether the adrenergic control of MCFP is mediated by adrenergic nerves and/or circulating catecholamines. In cod (Axelsson and Nilsson, 1986; Butler et al., 1989; Smith et al., 1985) and trout (Smith, 1978) it has been demonstrated that the increase in arterial blood pressure observed during moderate exercise is exclusively mediated by adrenergic nerves. Whether the same is true for the venous circulation in fish is not yet known.

A possible consequence of a decreased venous capacitance, manifested as the increase in MCFP, is that blood volume from the venous compartment is redistributed to other parts of the circulation, such as muscle capillary beds, respiratory organs and central veins (Pang, 2001). It is possible that much of the blood redistributed from the venous system in the sea bass during exercise, in addition to increasing cardiac preload, served to fill gill vasculature and muscle capillary beds. In mammals the splanchnic circulation has a high capacitance and is the primary reservoir for blood volume mobilization during exercise (Pang, 2001; Rothe, 1986). To what extent splanchnic venous blood volume is mobilized during exercise in fish is unclear, even though Stevens and Randall (1967) demonstrated that blood flow in the subintestinal vein (e.g. portal vein) decreased and venous pressure increased in rainbow trout. Albeit somewhat meager evidence, the observations are consistent with blood volume mobilization from the splanchnic venous compartment during exercise in fish. As judged from the drop in P_{DA} and R_{sys} during swimming at $2\ BL\ s^{-1}$ after prazosin, it is possible that the gut circulation continued to be perfused (unlike the normal decrease with exercise) as perfusion of locomotory muscles increased (Axelsson and Fritsche, 1991; Axelsson et al., 2000; Farrell et al., 2001; Thorarensen et al., 1993). Further research in this area is clearly needed.

Heart rate versus *stroke volume regulation during exercise*

Increased $\dot{Q}$ observed after force feeding in sea bass was due primarily to tachycardia (Axelsson et al., 2002). Similarly, in the present experiments, sea bass increased $\dot{Q}$ through tachycardia with no significant change in V_s, despite the fact that cardiac preload increased significantly (Fig. 3, Table 1). This shows that an increased cardiac preload does not

necessarily lead to an increased V_s in fish, but may instead compensate for a reduced cardiac filling time associated with an increase in f_H. Thus, within the scope of the present exercise challenge, $\dot{Q}$ in sea bass was frequency regulated.

Other studies on fish with various life-strategies also suggest that control of $\dot{Q}$ by f_H during exercise, might be more important than previously thought (Farrell, 1991; Farrell and Jones, 1992; Jones and Randall, 1978). Korsmeyer et al. (1997) found in the highly active yellowfin tuna (*Thunnus albacares*) that $\dot{Q}$ increased by 13.6% during exercise at 24°C exclusively through tachycardia and, in fact, V_s decreased. Similarly, during forced swimming at 0°C the Antarctic borch (*Pagothenia borchgrevinki*) increased $\dot{Q}$ by 75% by doubling f_H (Axelsson et al., 1992). Furthermore, Cooke et al. (2003) investigated the relative contribution of V_s and f_H to maximum cardiac output at 3°C in three North American species with various degrees of winter quiescence. Largemouth bass (*Micropterus salmoides*), a winter inactive species, increased $\dot{Q}$ by means of a 124% increase in f_H with a 24% reduction in V_s. Similarly, the intermediately active black crappie (*Pomoxis nigromaculatus*) increased $\dot{Q}$ by a 156% increase in f_H with a 56% reduction in V_s. By contrast, in the winter active white bass (*Morone chrysops*) maximum $\dot{Q}$ was attained by a 45% increase in f_H and a 55% increase in V_s. Within a species, temperature may modulate the relative contributions of f_H and V_s during exercise. For example, for maximum $\dot{Q}$ at 5°C and 10°C in largescale suckers (*Catostomus macrocheilus*), increased V_s contributed 58% and 62%, respectively (Kolok et al., 1993), whereas at 16°C f_H contributed 70% of maximum $\dot{Q}$ during exercise. Altogether these results indicate the importance of frequency regulation of $\dot{Q}$ in various fish species under various conditions but, of course, contrasts with several studies (mainly on salmonids) where increased V_s was the major means of increasing $\dot{Q}$ during exercise (Dunmall and Schreer, 2003; Farrell, 1991; Farrell and Jones, 1992; Jones and Randall, 1978; Kiceniuk and Jones, 1977).

Under the present experimental conditions, exercising sea bass used only tachycardia to increase $\dot{Q}$, but to what degree V_s might be modulated in sea bass at higher swimming speeds and different temperatures awaits further study. By performing the study at 22°C, which is in the upper range of the temperature preferendum for sea bass (Claireaux and Lagardere, 1999), it is possible that at lower water temperatures V_s could increase during swimming. Another concern is that fish only swam to 2 $BL\ s^{-1}$ and this resulted in a relatively small increase in $\dot{Q}$ (38%). Therefore, it is possible that at higher swimming speeds further increases in $\dot{Q}$ could occur through increased V_s.

In conclusion, this is the first study to measure variables related to venous return and cardiac filling pressure during exercise and to provide evidence for an active involvement of the venous vasculature during exercise in any species of fish. An α-adrenoceptor mediated control system was partially responsible for a decrease in venous capacitance, as reflected as an increase in MCFP in the swimming sea bass. This control would serve to: (1) maintain or increase the pressure gradient for venous return, thus matching venous return and cardiac output; (2) Increase central venous blood volume, and consequently cardiac preload; and (3) Possibly redistribute venous blood to other parts of the circulation, such as the gills and muscle capillaries. These results highlight the fact that an increased cardiac preload does not necessarily result in an increased V_s, but can instead compensate for the reduced filling time when f_H is increased, thereby offsetting potential decreases in V_s. In fact, under the present conditions, the exercise-induced increase in $\dot{Q}$ in sea bass was exclusively mediated by tachycardia.

Symbols

f_H	heart rate
M_b	body mass
MCFP	mean circulatory filling pressure
P_{CV}	central venous pressure
P_{DA}	dorsal aortic blood pressure
ΔP_V	pressure gradient for venous return
$\dot{Q}$	relative cardiac output
R_{sys}	total systemic resistance
R_v	resistance to venous return
U_{crit}	critical swimming speed
V_s	stroke volume

We are delighted to acknowledge Kenneth R. Olson for valuable comments on the manuscript and David J. McKenzie who kindly lent us the swim tunnel used in these experiments. E.S. and M.A. were supported by the Swedish research council (VR). A.P.F. was supported by NSERC Canada. J.A. was supported by a travel grant from the Swedish research council (VR). Financial help by IFREMER (G.C.) is also acknowledged.

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