Effects of Temperature on Cardiac Function in Teleost Fish

A.L. Kalinin, M.J. Costa, F.T. Rantin, and M.L. Glass

Abstract Changes of environmental temperatures immediately and substantially influence cardio-vascular function in teleost fish. In most species, the body temperature closely parallels that of the environment. Some species live at a nearly constant temperature, such as deep seas and polar oceans. On the other hand, subtropical and tropical environments expose fish to ever-changing temperatures, which provokes large adjustments of physiological and biochemical rate processes and behavioral patterns. Teleost fish display large differences in cardiac function, depending on adaptations to temperature, mode of life, and activity levels. Seasonal and acute environmental temperature fluctuations, and the temperature-dependent regulation of cardiac performance become crucial for ectothermic fish. This motivates a growing concern about the possible impacts of global warming, since it is associated with ever-changing temperatures and oxygen levels in aquatic ecosystems. Currently, there is a growing need to understand the physiological basis of biodiversity, and to evaluate the resistance of distinct species to cope with changing environments. On this background, this chapter reviews how ambient temperature changes cardiac function in teleost fish, emphasizing both in vivo (heart rate, stroke volume and cardiac output) and in vitro components (cardiac contractility).

1 Introduction

Teleost fish are by far the largest number of species, when compared to other groups of vertebrates. In the oceans, they are dominant and invade an amazing number of different marine habitats, and the freshwater habitats are also dominated by teleosts (Nelson 1994). The evolutionary adaptation to different habits, modes of life and activity levels has resulted in an impressive interspecific variation

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with regard to cardiac anatomy and physiology which, in particular, concerns excitation–contraction coupling (E–C coupling).

Living in a variety of vulnerable habitats, teleosts are increasingly threatened by global warming. Greenhouse gases released mainly by human activities such as burning of coal, oil, and natural gases have recently caused a considerable increase in mean global temperatures. Against this background, most studies predict that ambient temperatures will increase by 1.4–5.8°C from now until the end of the century, increasing the temperatures of oceans and inland waters. Increased water temperatures, as well as changes in rainfall, oceanic currents, and sea levels are already affecting the world's fish and fisheries (Combes 2005).

In most species of fish, the body temperature closely parallels that of their environment. Some species live at a nearly constant temperature, and these include deep seas or polar oceans, while other species are exposed to ever-changing temperatures, which provokes major changes in their physiological and biochemical rate processes and behavioral patterns. Depending on adaptations to temperature, life styles, and activity levels, teleost fish display large differences in cardiac function. When exposed to seasonal and acute environmental temperature fluctuations, the temperature-dependent regulation of cardiac contractility becomes crucial for ectothermic fish. For example, vertical movement of fish in the water column across a thermocline can cause significant and rapid oscillations of body temperature. Since these changes are, largely, unpredictable, the fish heart must have some intrinsic mechanism to protect against acute temperature changes (Tiitu 2002; Vornanen et al. 2002; Rocha et al. 2007a, b).

There is a growing concern about the possible impacts of global warming on fish, because it is associated with changes in temperature and oxygen levels in all aquatic ecosystems. Currently, there is a growing need to understand the physiological basis of biodiversity, and to evaluate the resistance of distinct species to withstand changes of the environment. Against this background, this chapter reviews current information on the effects of ambient temperature changes, with a special emphasis on cardiac function in teleost fish.

1.1 Temperature as a Controlling and Limiting Factor

Temperature range is one of the most important features to describe any water body, because the temperature influences the development and distribution of the species. In addition, the degree of circulation will disperse nutrients and pollutants within the water column. Moreover, temperature affects virtually all levels of biological organization, from molecular diffusion to biochemical reactions, membrane function and organ function, which constitute the whole organism.

According to Guderley and St-Pierre (2002), ectothermic vertebrates have two choices when exposed to unfavorable temperatures. They may move to a compatible temperature or, alternatively, remain within their habitat. In this latter case, they will be exposed to the constraints imposed by the law of Arrhenius, which reflects

the impact of temperature upon the frequency of molecular collisions and, in addition, formally describes the thermal dependence of rate processes. When subjected to environmental temperature changes, organisms can: (a) submit to the effects of Q_{10} by slowing down or, alternatively, accelerating their physiological processes, (b) enhance the Q_{10} effects on rate processes (hibernation, torpor), or (c) offset the Q_{10} effects by triggering compensatory mechanisms to maintain functions and capacities. Additionally, a combination of high temperatures and unfavorable conditions, such as hypoxia and decreased food availability, may lead to aestivation, which is a period of torpor.

Body temperatures of small animals can not deviate much from the temperature of the aquatic environment. This is due to the high thermal conductivity and large heat capacity of water (Schmidt-Nielsen et al. 1975; Stevens and Sutterlin 1976). Most teleost fish must cope with ambient temperature changes, which is an important aspect of their physiology. Nevertheless, highly active species of large aerobic scope such as blue fin tuna, skipjack tuna and bigeyed tuna are able to maintain an elevated body temperature in relation to their environment (Stevens et al. 1974; Stevens and Neill 1978).

A preference for a specific temperature range is a common feature among teleosts. This preferred temperature range generally coincides with an optimal growth rate (Jobling 1982) and/or with a high aerobic capacity (Kelsch and Neill 1990). Seasonal changes, diurnal rhythms and growth can, however, alter the preferred temperature in a species-specific manner (Reynolds and Casterlin 1979; Roberts 1979). Further, the preferred temperature is influenced by the O_2 availability, because fish faced with hypoxic conditions prefer lower temperatures, which decreases metabolic demands. Obviously, this response increases their survival rate, when in hypoxia (Schurmann and Christiansen 1994). In addition, animals inhabiting small water bodies such as shallow and small lakes, streams, ponds, canal and drains are subjected to diurnal or seasonal temperature changes without any major temperature gradient from surface to bottom, which sets very narrow limits for any efficient behavioral thermoregulation (Harder et al. 1999).

Cardiac function in eurythermal fish is subject to large temperature changes, and the effects of temperature acclimation on cardiac contractility vary according to the strategy of environmental adaptation (Aho and Vornanen 1999; Tiitu and Vornanen 2001, 2002a). Conversely, stenothermal species have acquired special characteristics, which are highly adapted to habitats with specific thermal characteristics. These specific evolutionary adaptations occurred, however, at the expense of reduced plasticity for temperature change (Tiitu and Vornanen 2002b). For instance, Egginton et al. (2006) pointed out that most of the organisms in the Antarctic marine environment are stenothermal and, therefore, will die at temperatures in the range +5–7°C, and such Antarctic species will be at high risk when faced with even moderate increases of ambient temperature, which may be triggered by global warming.

For the reasons mentioned, temperature can be classified as both a controlling and limiting environmental factor for fish, as earlier described by Fry (1948), which requires physiological and behavioral adjustments. In this review we discuss what

happens to cardiac function in teleost fish exposed to unavoidable changes of body temperature.

2 Cardiac Output, Heart Rate, and Stroke Volume

The function of the heart is to supply blood to the tissues, and generate and sustain the systolic blood pressure needed for adequate perfusion of the tissues. The contraction of the heart by its muscular walls must generate sufficient pressure to propel blood from the cardiac chamber (e.g., ventricle), into the ventral aorta. For each beat, a volume of blood is ejected. This stroke volume (S_V–ml kg^{-1}), times the number of beats per minute (heart rate, f_H–bpm), equals the cardiac output (Q), which is the total volume of blood delivered by the ventricle each minute (ml min^{-1} kg^{-1}). In this context, all changes in either f_H or S_V will influence the other components. In addition, Q is determined not only by activity of the heart but also by peripheral resistance (Klabunde 2004). Cardiac function is well established for mammals, whereas data on fish are more controversial, which results from a lack of standard methods (e.g., estimated or direct measured) and large interspecific differences (Table 1).

Changes of environmental temperatures immediately and substantially influence cardio-vascular function in teleost fish. For the majority of teleosts, f_H is typically between 10 and 60 bpm and will not exceed 120 bpm (Farrell 1991; Anelli-Jr et al. 2004). Nevertheless, even higher values for f_H were described by Brill (1987) who studied scombrid species, including yellowfin tuna, *Thunnus albacares* (90–160 bpm), kawakawa, *Euthynnus affinis* (175–253 bpm), and skipjack tuna, *Katsuwonus pelamis* (191–237 bpm), where high f_H and high swimming performance are matched. In this context, the frillfin goby, *Bathygobius soporator*, a resident intertidal species, is abundant in Brazilian rocky tide pools, where the temperature can reach more than 40°C during low tide. When exposed to acute temperature transitions from 25 to 35°C, this species increased f_H progressively and significantly, reaching a maximum value of about 225 bpm (Rantin et al. 1998).

The temperature coefficient Q_{10} provides a measure of the rate of change of a biological or chemical system, which increases temperature by 10°C. According to Webber et al. (1998), a Q_{10} of 1.0 would imply that metabolism is independent of temperature. The expected value for an increase is $Q_{10} = 2.0$, which implies that metabolism increases twofold for each increase by 10°C.

In most temperate fish, acute changes of temperature typically increase f_H with an average Q_{10} of about 2.0 (Priede 1974; Butler and Taylor 1975; Cech et al. 1976; Graham and Farrell 1985; Blank et al. 2002; Tiitu and Vornanen 2002a). In addition, Rantin et al. (1998) suggested that these Q_{10} values also apply to tropical species. Nevertheless, some tropical species present Q_{10} values for f_H of about 3.0 during acute temperature transitions. This applied to pacu, *Piaractus mesopotamicus*, an active migratory serrasalmid fish which increased f_H from 37 to 115 bpm (Anelli-Jr et al. 2004). This is compatible with values for trahira, *Hoplias*

Table 1 Cardiac output (Q), heart rate (f_H) and stroke volume (S_V) of different teleosts species at different temperatures and measurement techniques

Species	Temp (°C)	Wt (g)	Q ($ml\,kg^{-1}\,min^{-1}$)	f_H (bpm)	S_v ($ml\,kg^{-1}$)	Method	Reference
	6	~1,110	9.5	7	1.22	In vivo – Resting	
Cyprinus carpio	10	~1,230	19	12	1.58	In vivo – Resting	Stecyk and Farrell (2002)
	15	~790	15	17	0.77	In vivo – Resting	
	18	250–320	91	65–70	1.38	In situ perfused heart	Farrell et al. (2007)
Dicentrarchus labrax	22	250–320	95	95–100	1.05	In situ perfused heart	
	5	~1,910	9	16	0.52	In vivo – Resting	
	5	~1,910	31	28	1.12	In vivo – Swimming	Webber et al. (1998)
	10	~1,910	12	28	0.45	In vivo – Resting	
Gadus morhua	10	~1,910	35	40	0.88	In vivo – Swimming	
	10	861–1,335	22	36	0.60	In vivo – Swimming	Gollock et al. (2006)
	20	861–1,335	47	73	0.67	In vivo – Swimming	Gollock et al. (2006)
	25	1,600	138[b]	125.9	1.1	Spinally blocked	Bushnell and Brill (1992)
Katsuwonus pelamis	25	1,300	105[b]	154	0.68	In vitro perfused heart	Farrell et al. (1992)
	4	~1,120	5	38	0.14	In vivo – Resting	
	4	~1,120	14	44	0.25	In vivo – Swimming	
	11	~822	21	52	0.53	In vivo – Resting	
	11	~822	73	59	1.35	In vivo – Swimming	Taylor et al. (1996)
	18	~810	20	91	0.25	In vivo – Resting	
Oncorhynchus mykiss	18	~810	41	95	0.50	In vivo – Swimming	
	10	~500	15	55	0.27	In situ perfused heart	Overgaard et al. (2004)
	10	290–1,060	100[a]	47	100[a]	In vivo – Resting	
	13	290–1,060	120[a]	62	91[a]	In vivo –Resting	Sandblom and Axelsson (2007)
	16	290–1,060	131[a]	76	80[a]	In vivo – Resting	

(*Continued*)

Table 1 (Continued)

Species	Temp (°C)	Wt (g)	Q ($ml\,kg^{-1}\,min^{-1}$)	f_H (bpm)	S_v ($ml\,kg^{-1}$)	Method	Reference
	4	453–843	9,8	21	0.5	In vivo – Resting	
	4	453–843	25.4	31.6	0.80	In vivo – Swimming	Joaquim et al. (2004)
Pleuronectes americanus	10	453–843	15.5	34	0.5	In vivo – Resting	
	10	453–843	39.2	52.4	0.74	In vivo – Swimming	
Thunnus alalunga	16.5	9,100	28[b]	87	0.32	Anesthetized	Lai et al. (1987)
	21.9	9,300	44[b]	115	0.38	Anesthetized	White et al. (1988)
Thunnus albacares	25	1,400	126[b]	96.7	1,3	Spinally blocked	Bushnell and Brill (1992)
	25	1,400	75[b]	112	0.67	Anesthetized	Jones et al. (1993)
	5	~6,700	19	23	0.86	In situ perfused heart	
	10	~6,700	21	36	0.57	In situ perfused heart	
Thunnus orientalis	15	~6,700	20	55	0.37	In situ perfused heart	Blank et al. (2004)
	20	~6,700	17	78	0.23	In situ perfused heart	
	25	~6,700	18	100	0.18	In situ perfused heart	

[a] Data as a % of initial values at 10°C; [b] Data calculated based on f_H and S_v values

Table 2 Q_{10} values for resting heart rate (f_H–*bpm*) during temperature changes for different tropical, temperate and polar teleost species

Species	°C	Q_{10}	Reference
Bathygobius soporator	25–5	1.6	Rantin et al. (1998)
Chaenocephalus aceratus	2 to −1.8	6.3	Bastos-Ramos et al. (1998)
Gadus morhua	5–10	2.9	Webber et al. (1998)
Hoplias malabaricus	25–35	3.0	Olle 2003
	5–15	1.4	
Leiopotherapon unicolor	15–25	1.2	Gehrke and Fielder (1988)
	25–35	1.1	
Notothenia neglecta	2 to −1.8	4.8	Bastos-Ramos et al. (1998)
Oreochromis niloticus	25–35	2.4	Costa et al. (2000)
	−1 to 3	3.3	
Pagothenia borchgrevinki	3–6	2.5	Lowe et al. (2005)
	6–10	1.2	
Piaractus mesopotamicus	25–35	3.1	Anelli-Jr et al. 2004
	4–10	2.2	Joaquim et al. (2004)
Pleuronectes americanus	5–10	1.4	Cech et al. (1976)
Prochilodus lineatus	25–35	2.3	Kalinin, unpublished data
Synbranchus marmoratus	25–35	1.6	Rocha et al. (2007a)
	25–15	2.3	
Thunnus albacares	10–20	3.6	Blank et al. (2002)
	15–25	2.6	
Thunnus orientalis	5–15	2.4	Blank et al. (2004)
	10–20	2.1	
	15–25	1.8	

malabaricus, which is a sedentary erythrinid species that may increase f_H from 35 to 105 bpm (Olle 2003) if exposed to acute temperature transitions from 25 to 35°C. Surprisingly, this also applied to the Antarctic notothenioid *Pagothenia borchgrevinki,* which increased f_H from 16 to 33.7 bpm during temperature transitions from −1 to 6°C (Lowe et al. 2005). This response seems large in relation to the extremely low temperature of the environment. Table 2 presents selected Q_{10} values for f_H, underlying the considerable variability among species.

Taken together, these results suggest that increases in f_H can be the main adjustment triggered to maintain adequate cardiac performance during increases of temperature. Changes of f_H are considered most important for modulations of cardiac output, which has also been described for the tuna heart by Farrell (1996) and Blank et al. (2002). As to low temperatures, Webber et al. (1998) recorded Q_{10} values for resting values of Q and f_H in cod (*Gadus morhua*). As calculated from 5 to 10°C, the value was 1.65 for Q and 2.93 for f_H. These authors concluded that the reduction in Q with lower temperature can be attributed to considerable reductions of f_H (Armstrong 1986; Gehrke and Fielder 1988; Kolok et al. 1993; Keen and Farrell 1994; Claireaux et al. 1995).

Elevated temperatures increase the depolarization frequency of the pacemaker cells and, consequently, of the heart rate (for a review, see Tibbits et al. 1992a). In addition, higher temperatures shorten the duration of the ventricular action

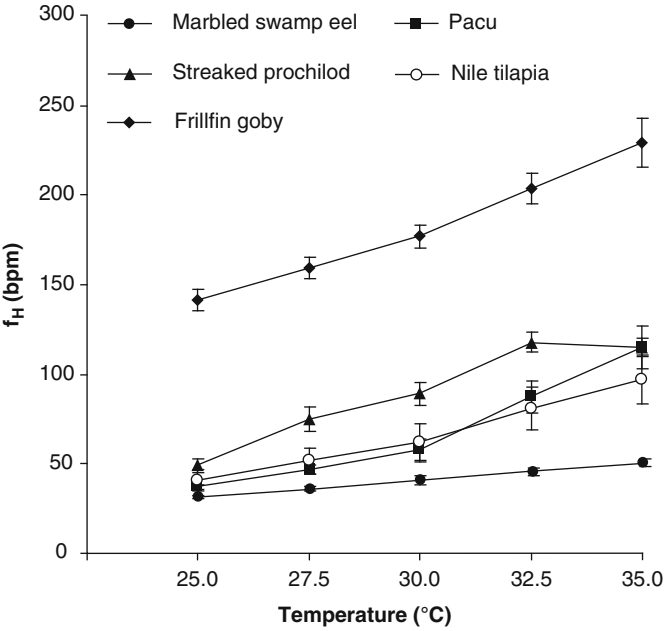

Fig. 1 Effects of temperature on heart rate (f_H) for Nile tilapia, *Oreochromis niloticus* (Costa et al. 2000), frillfin goby, *Bathygobius soporator* (Rantin et al. 1998), pacu, *Piaractus mesopotamicus* (Anelli-Jr et al. 2004), marbled swamp eel, *Synbranchus marmoratus* (Rocha et al. 2007a), and streaked prochlilod, *Prochilodus lineatus* (Kalinin, unpublished data), acclimated to 25°C

potential (VAP_D) (Lennard and Huddart 1991; Aguiar et al. 2002). This effect can be attributed to an increased permeability of the sarcolemma (SL), leading to an earlier final repolarization. Consistent with these temperature effects, several tropical species increase f_H when exposed to high temperatures (Fig. 1).

In 1988, Gehrke and Fielder combined increases in temperature and different oxygen levels to analyze the cardiorespiratory responses of the spangled perch, *Leiopotherapon unicolor.* Widespread throughout inland waters of Australia, this species survives temperatures from 5 to 44°C and dissolved oxygen concentrations down to $1\,mg\,L^{-1}$. The purpose was to simulate steep thermal gradients without any acclimation. The f_H of *L. unicolor* increased in response to rising temperature, and decreased as the temperature became reduced (Fig. 2). At 100% O_2 saturation of the water, f_H reached a maximum mean rate of 104.1 bpm at 35°C, compared to the minimum mean rate of 12.2 bpm at 5°C, which gives an overall Q_{10} value of 2.85. Q_{10} values for f_H at other oxygen tensions ranged from 2.50 to 3.05. Progressive hypoxia caused bradycardia and heart rate became dependent on available oxygen before oxygen consumption declined, with the exception of individuals at 35°C.

Little information is available on the in vivo effects of temperature on cardiorespiratory systems, whereas substantial information has been provided by in vitro

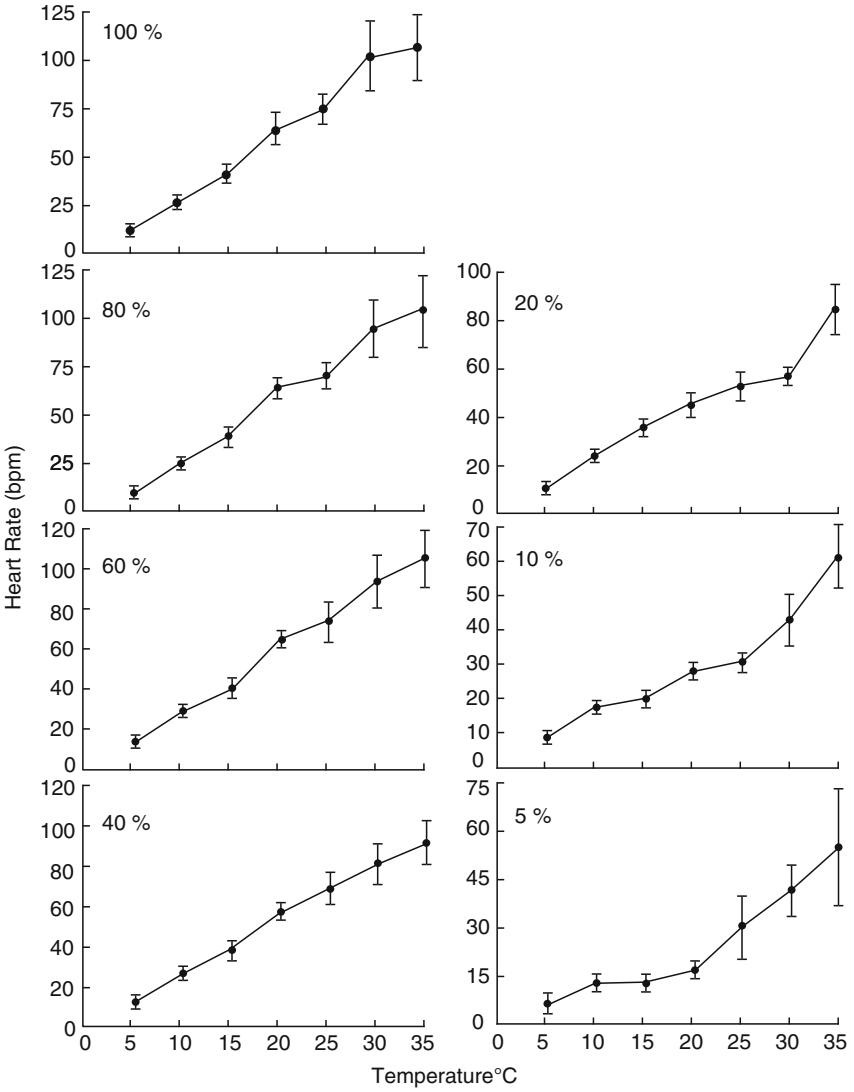

Fig. 2 Heart rate (f_H) of splangled perch, *Leiopotherapon unicolor*, in response to temperature at levels of oxygen saturation from 100% to 5% (from Gehrke and Fielder 1988)

studies on perfused hearts (Farrell 2002). Furthermore, the majority of the studies were conducted with temperate fish species.

Several in vitro studies have demonstrated an inverse relationship between f_H and V_S in fish hearts in response to acute temperature changes (Graham and Farrell 1985; Webber et al. 1998; Yamamitsu and Itazawa 1990; Farrell 2002).

Studying the lincod, *Ophiodon elongates*, Stevens et al. (1972) pointed out that changes of f_H are most important for modulation of Q, in vivo, while changes in

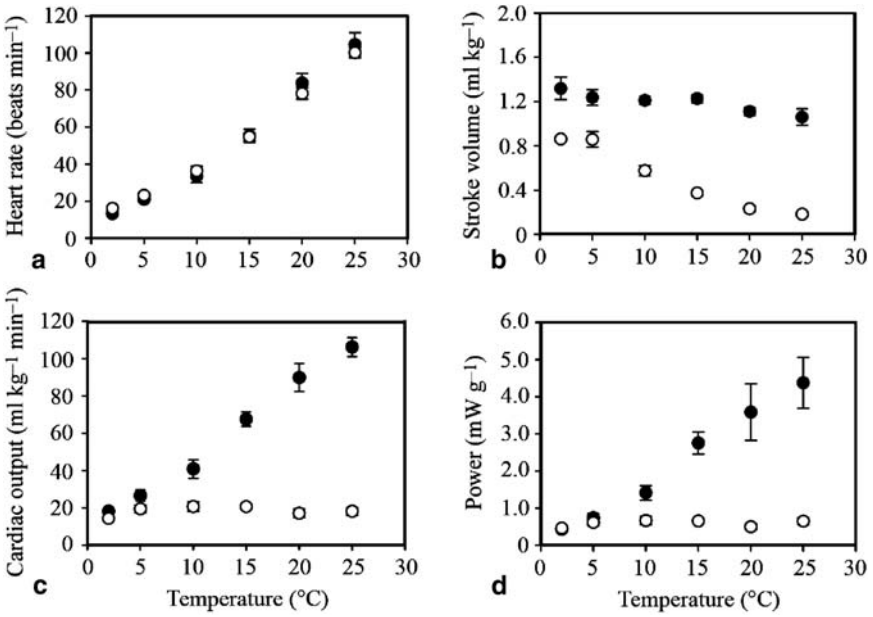

Fig. 3 Values of cardiac parameters recorded from spontaneously beating yellowfin tuna hearts *in situ* at temperatures of 10–25°C: heart rate (**a**), stroke volume (**b**), cardiac output (**c**), myocardial power output (**d**). Standard conditions are shown as *open symbols*, maximal conditions as *filled symbols*. (from Blank et al. 2002)

V_S are of minor importance. Blank et al. (2002) evaluated the effects of acute temperature changes on cardiac function of the in situ-perfused heart preparation of yellowfin tuna (*Thunnus albacares*), and they found that f_H became reduced in a linear manner, falling from ~106 bpm at 25°C to ~20 bpm at 10°C. Meanwhile, S_V increased as temperature fell, resulting in a very limited effect on cardiac output under standard conditions (Fig. 3). Based on these results, and earlier in vitro experiments (Freund 1999), it was proved that changes in S_V are, very probably, important for maintained cardiac output in tuna confronted with changing temperatures (cf. Farrell et al. 1996). Consistently, an increase in V_S (Webber et al. 1998) partially compensated for the large reduction in f_H from 10 to 5°C recorded in cod.

Joaquim et al. (2004) conducted the first direct measurements of resting and maximum cardiac function in winter flounder, *Pleuronectes americanus,* determining the critical swimming capacity at 4 and 10°C in relation to temperature. In resting fish, they found a temperature-induced increase in Q (from 9.8 to 15.5 ml kg^{-1} min^{-1}), exclusively achieved by an elevated f_H (from 20.5 to 34.3 bpm – Q_{10} of 2.35), while resting S_V was independent in relation to acclimation temperature (0.50 at 4°C and 0.47 ml kg^{-1} at 10°C)P. Previously, Cech et al. (1976) studied the same species, and reported a significantly lower Q_{10} (1.36) for f_H between 5 and 10°C. This could be due to a higher resting f_H at the lower temperature (30 bpm) combined with an increase in S_V from 0.55 ml kg^{-1} at 5°C to 0.90 ml kg^{-1} at 15°C. According to

Joaquim et al. (2004), the Sv values obtained by Cech et al. (1976) were calculated from Q values based on the Fick equation. This calculation does not include temperature effects on cutaneous O_2 uptake, and it is possible that the reported S_V increases resulted from temperature effects on cutaneous oxygen uptake. The authors emphasized that their results on winter flounder differed from those of Kolok et al. (1993), who studied the largescale sucker, *Catostomus macrocheilus*, in which Q failed to increase, when acclimated to temperatures of 5–10°C. This occurred because a 25% increase in f_H (Q_{10} of 1.65) was counteracted by 30% decrease in S_V. These intraspecific differences suggest that Q of some species may be rather insensitive to some segments of temperature (Joaquim et al. 2004).

As exposed above, it is clear that alterations in teleosts' heart function caused by temperature changes are highly variable, as shown in Tables 1 and 2. In addition, the methodological differences combined with interspecific characteristics and thermal acclimation exerts a pronounced effect on cardiac parameters.

Matikainen and Vornanen (1992) recorded f_H in response to acute increases of temperature in the crucian carp (*Carassius carassius*) acclimated to 5°C and 15°C (Fig. 4). The responses were identical at the lowest temperatures (2 or 4°C), but above this range f_H was higher in warm-acclimated summer fish, when compared to cold-acclimated winter fish. According to the authors, this type of temperature acclimatization, where the cold-acclimated animal has the lower Q_{10} value, is designated as noncompensatory or inverse acclimation by Prosser (1973).

Likewise, specimens of Nile tilapia, *Oreochromis niloticus*, acclimated to 35°C and then submitted to acute temperature reductions down to 15°C, had a higher f_H at 15°C and 20°C than the cold-acclimated (15°C) specimens submitted to

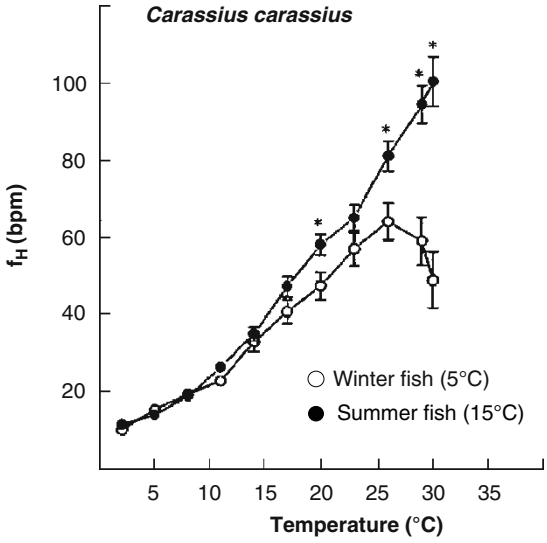

Fig. 4 Temperature dependence of in vivo heart rate (f_H) in crucian carp, *Carassius carassius*, during summer and winter (from Matikainen and Vornanen 1992)

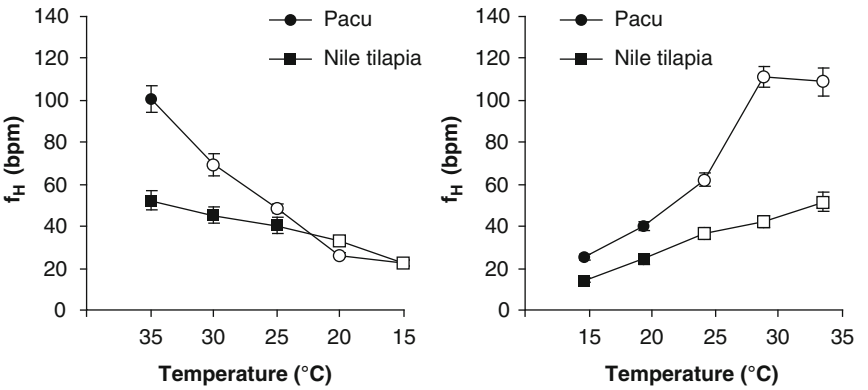

Fig. 5 The effects of temperature on heart rate (f_H) for Nile tilapia, *Oreochromis niloticus* (Maricondi-Massari et al. 1998), and pacu, *Piaractus mesopotamicus* (Aguiar et al. 2002) acclimated to 35°C (*left panel*) and 15°C (*right panel*). *Open symbols* indicate a significant difference ($p < 0.05$) in relation to the initial values

temperatures up to 35°C (Fig. 5) (Maricondi-Massari et al. 1998). On the other hand, Aguiar et al. (2002) reported significantly higher values of f_H in cold-acclimated (15°C) pacu, *P. mesopotamicus*, when compared to the warm-acclimated (35°C) group (Fig. 5). Similarly, goldfish, *Carassius auratus*, acclimated to 10°C displayed a higher f_H than the specimens acclimated to 25°C (Morita and Tsukuda 1995). Likewise, Seibert (1979) recorded a lower f_H for warm-acclimated European eel, *Anguilla anguilla*, when compared to the cold-adapted eels at all temperatures, and this difference could be abolished by vagal blockade. Similar results were also obtained for medaka, *Oryzaias latipes*, guppy, *Lebistes reticulatus* (Tsukuda 1961) and the sole, *Solea vulgaris* (Sureau et al. 1989).

Taken together, these results indicate that the cardiac alterations following temperature changes are not uniform among teleosts, and the effects of temperature acclimation on f_H are rather species-specific, reflecting seasonally induced changes in the temperature sensitivity of the pacemaker cells.

As in other vertebrates, the f_H of teleost fish is determined both by the intrinsic rate of pacemaker cells in the sinus venosus and the extrinsic control by the autonomic nervous system along with humoral factors (Laurent et al. 1983), and temperature affects both intrinsic and extrinsic components. According to Haverinen and Vornanen (2006), low temperatures slow down f_H, which tends to decrease cardiac output as well as the level of activity of ectothermic animals, in particular within habitats of large seasonal temperature changes. Several fish species of the north temperate latitudes such as rainbow trout (*Oncorhynchus mykiss*), perch (*Perca fluviatilis*), European eel (*A. Anguilla*), and sole (*S. vulgaris*), are able to oppose or avoid the effects of cold temperatures on f_H, which occurs by long-term mechanisms that are triggered upon exposure to low temperature. These authors suggest that such compensatory mechanisms involve a cold-induced decrease in the

inhibitory cholinergic control of the pacemaker rate and are likely to involve the pacemaker mechanism itself.

Teleost hearts usually operate under some degree of vagal tone, which varies due to physiological and environmental conditions. The extent of sympathetic cardio-acceloratory innervation in teleosts remains unclear, but some species possess adrenergic nerve fibers, and an adrenergic tonus has been verified in vivo, but the relative importance of neuronal versus humoral influences has not been evaluated. Increased ventricular force contraction and f_H (positive inotropy and chronotropy) are mediated by β-adrenoreceptors on pacemaker cells and myocytes, whereas α-adrenoreceptors mediate negative chronotropy (Egginton et al. 2006).

According to Seibert (1979), vagal tone increases with temperature in the eel (*A. anguilla*), and this also seems to be the case in pacu (*P. mesopotamicus*) described by Aguiar et al. (2002), as shown in Fig. 5. However, in trout and carp parasympathetic control dominates at low temperatures, with the sympathetic control of f_H assuming a greater importance at higher temperatures (Farrell 1984). In the notothenioid *P. borchgrevinki*, an increased f_H during temperature transitions from −1 to 6°C was due to a 45% increase in excitatory adrenergic tone, masking a 37% increase in inhibitory cholinergic tone (Lowe et al. 2005). Additionally, Egginton et al. (2006) emphasized that, in this species, acclimation to a high temperature resets resting f_H to a lower level, which indicates an increased vagal inhibition (Seebacher et al. 2005). These findings reinforce the absence of uniform chronotropic responses to temperature acclimation in teleosts.

2.1 Heart Pacemaker

Unfortunately, the size and exact location of the primary pacemaker area of the fish heart are incompletely defined and, according to Satchell (1991), the pacemaker location within the fish heart is not uniform. In carp, *Cyprinus carpio* (Saito 1973), and Japanese loach, *Misgurnus anguillicaudatus* (Yamauchi et al. 1973), the primary pacemaker area has been located to the sinoatrial junction. In trout, Haverinen and Vornanen (2007) exclusively recorded primary pacemaker potentials from the base of the sinoatrial valve, where a morphologically distinct ring of tissue comprising myocytes and neural elements was detected by histological methods (Fig. 6).

According to Haverinen and Vornanen (2007), the diastolic depolarization of the pacemaker cells, which elicits the regular firing of pacemaker action potentials (APs), results from a combined action of many sarcolemmal ion currents, including T- and L-type Ca^{2+} current, slow and fast components of the delayed rectifier K^+ current, transient outward K^+ current, inward Na^+ current, and the pacemaker current carried by both K^+ and Na^+ ions. They suggest that the pacemaker mechanism could involve a close interplay between Ca^{2+}-induced Ca^{2+} release (CICR) of the sarcoplasmic reticulum (SR) and sarcolemmal Na^+/Ca^{2+} exchange (NCX), since subsarcolemmal SR Ca^{2+} release can accelerate diastolic depolarization via inward

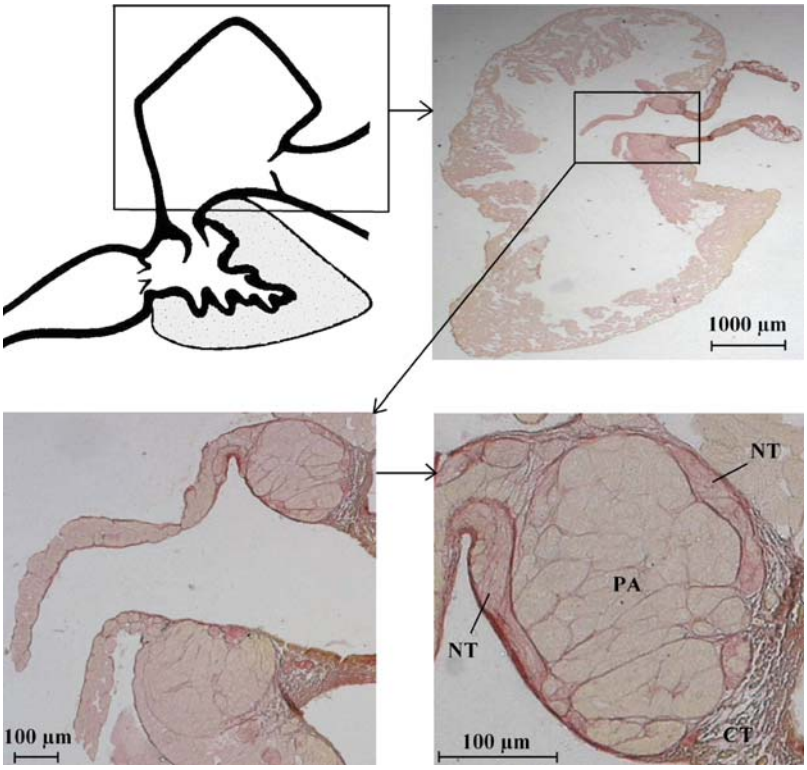

Fig. 6 Light microscopic histology showing structure and location of the sinoatrial nodal tissue of the rainbow trout heart. *Top left panel*: a schematic presentation of the heart structure and the area for histological inspection (within the *box*). *Top right panel*: a photomicrograph of the trout atrium and the adjacent part of the sinus venosus. The junctional area between sinus venosus and atrium, including the sinoatrial valve, is shown framed in the *box*. *Bottom left panel*: a closer look at the nodal tissue at the base of the sinoatrial valve. *Bottom right* panel: an enlarged image of the sinoatrial nodal tissue. *CT*, connective tissue; *NT*, nervous tissue; *PA*, pacemaker area (from Haverinen and Vornanen 2007)

NCX current. The mechanisms involved in cardiac Ca^{2+} management and related effects of temperature will be discussed in the in vitro section of this chapter.

Previous studies have shown that the inherent rhythmicity of pacemaker activity of the marine teleost plaice (*Pleuronectes platessa*) measured in vitro has a high temperature coefficient (Harper and Watt 1990; Windram et al. 1993). Furthermore, Harper et al. (1995) reported that temperature acclimation results in compensatory changes, so that pacemaker rhythms in cold-acclimated fish are higher, when compared to their warm-acclimated counterparts. At least in the plaice, the results show that the temperature coefficient of discharge activity of the cardiac pacemaker tissue displays seasonal variations. Accordingly, annual changes would imply that f_H of winter plaice would be maintained at a rate similar to that of the summer season.

Working on trout, Haverinen and Vornanen (2007) reported that the intrinsic beating rate of the sinoatrial pacemaker was higher in cold-acclimated (46 ± 6 APs min^{-1}) than in warm-acclimated fish (38 ± 3 APs min^{-1}), and similar responses were obtained from isolated pacemaker cells (44 ± 6 vs. 38 ± 6 APs min^{-1}), which corroborates the hypothesis that thermal acclimation modifies the intrinsic pacemaker mechanism of fish heart.

Unfortunately, information is very limited and only temperate species have been studied, which makes conclusions difficult concerning the effects of acclimation on the pacemaker activity. Nevertheless, some techniques, e.g., electrocardiography, can provide some information on the electrical activity of heart cells.

2.2 ECG

As discussed above, temperature strongly influences the contractile performance of cardiac muscle and the discharge rate of pacemaker cells, but the mechanisms of wave pattern variability in ectothermic vertebrates are incompletely understood, and data are few concerning the electrical properties of fish heart in vivo and in vitro.

One of the methods employed to characterize the state of the heart is electrocardiography. Cardiac alterations are extensively used for clinical diagnosis in human cardiology, while the mentioned methods have been little used for the analysis of cardiac function in fish, which probably relates to difficulties in obtaining consistent ECG patterns.

A detailed analysis of cardiac electric activity requires several ECG leads, sufficient to detect each component of the ECG waves and to establish the electrical axis of the heart. However, the use of several leads in fish is difficult for several reasons, including the anatomic diversity of these animals and, in particular, a lack of information on the best sites to place the electrodes in relation to the heart. In this respect, the ECG pattern depends on body shape and should be established for each species. In addition, most of the studies on fish electrocardiography have been performed on anesthetized fish and/or under other stressing conditions that influences the ECG. Nevertheless, important information on myocardial function can be obtained using a single lead provided that the procedure is well standardized for the studied species (for a review, see Satchell 1991; Rantin et al. 1995). The electrocardiogram (ECG) of fish presents the basic electrical events known from mammalian ECG, i.e., the P wave represents the atrium depolarization, the QRS complex indicates the activation of the ventricle, and the T wave corresponds to the ventricular repolarization (Rantin et al. 1995).

Studies conducted with two tropical species, the Nile tilapia, *O. niloticus* (Maricondi-Massari et al. 1998), and pacu, *P. mesopotamicus* (Aguiar et al. 2002) have shown that neither thermal acclimation nor increases or decreases in temperature affected the QRS and the T wave amplitudes (Fig. 7). These results are consistent with Morita and Tsukuda (1995), who studied QRS complexes of goldfish acclimated to 10°C and 25°C. Based on standard electrocardiography, the

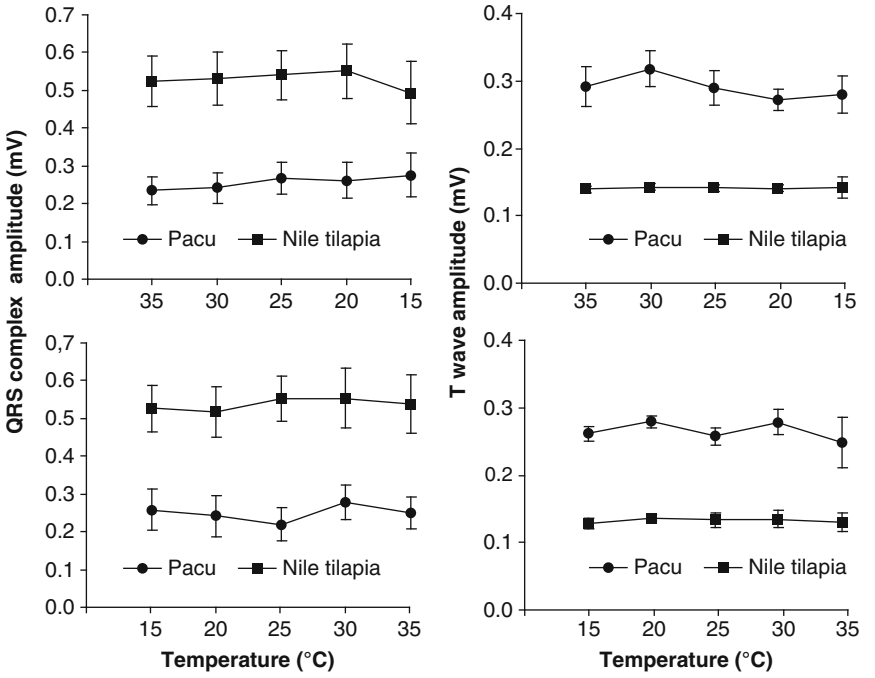

Fig. 7 Effects of temperature on the QRS and T wave amplitudes for Nile tilapia, *Oreochromis niloticus* (Maricondi-Massari et al. 1998), and pacu, *Piaractus mesopotamicus* (Aguiar et al. 2002)

projection of the depolarization and repolarization vectors on the derivation line of the ECG provides the amplitudes of the QRS and T waves respectively. Thus, the ECGs indicated that, in these species (goldfish, Nile tilapia and pacu) the directions of the ventricular depolarization and, moreover, repolarization remained constant, in spite of large variations of temperature. Further, both QRS complexes and T waves recorded for *O. niloticus* and *P. mesopotamicus* were consistently positive, indicating that the ventricular repolarization and depolarization followed the same direction.

According to Cotter and Rodnick (2006), the QRS complex duration indirectly expresses the conduction velocity of electrical signals through ventricular tissue, which is inversely related to f_H during and after exercise in healthy mammals. The precise control of QRS duration is not known, but is probably modulated by autonomic tone and possibly other factors (Trautwein 1963; Goldberger and Bhargava 1983; Nakagawa et al. 2000; Cotter and Rodnick 2006).

In warm-acclimated *O. niloticus*, its QRS duration increased progressively during stepwise increments of temperature, while the QRS duration only increased at 15°C in *P. mesopotamicus*. In the cold-acclimated groups, QRS duration was significantly lower at 20, 25, 30 and 35°C for O. niloticus, and at 30 and 35°C for *P. mesopotamicus* (Fig. 8). These results indicate that the depolarization processes were less sensitive to temperature changes in *P. mesopotamicus* than in

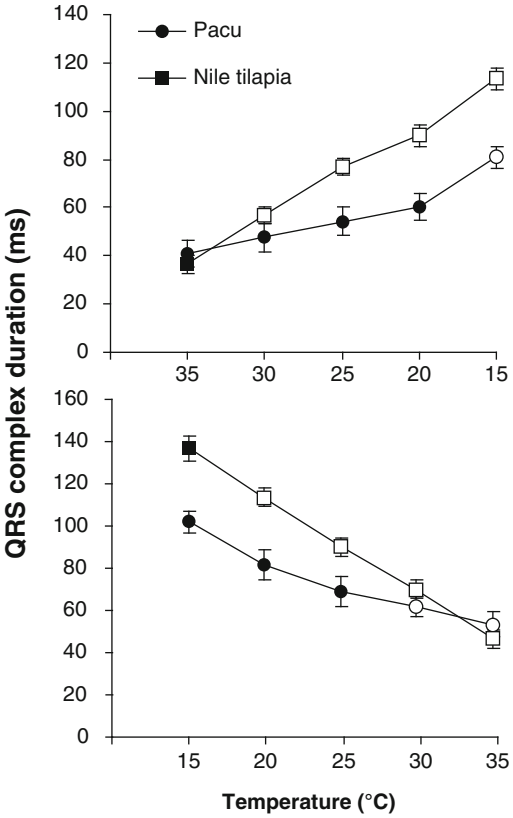

Fig. 8 Effects of temperature on the QRS complex duration for Nile tilapia, *Oreochromis niloticus* (Maricondi-Massari et al. 1998), and pacu, *Piaractus mesopotamicus* (Aguiar et al. 2002) acclimated to 35°C (*upper panel*) and 15°C (*lower panel*). *Open symbols* indicate a significant difference ($p < 0.05$) in relation to the initial values

O. nilotucus. Additionally, in spite of the significantly higher values presented by the warm-acclimated fish, *P. mesopotamicus* increased or decreased the T wave duration with the same magnitude (Fig. 9).

A segment in an electrocardiogram is the region between two waves: the ST segment is the isoelectric line followed by the QRS, which terminates at the onset of the T wave, and represents the time at which the entire ventricle is depolarized and roughly corresponds to the *plateau* phase of the ventricular action potential. The ST segment is important in the diagnosis of ventricular ischemia or hypoxia because, under those conditions, the ST segment can either become depressed or elevated (Klabunde 2004). Temperature changes also have a considerable effect on ST segment, as shown in Fig. 10. The magnitude of this effect seems to be species-specific since the Q_{10} for ST segment in *P. mesopotamicus* is about 3 while *O. niloticus* does not exceed 2, irrespective of temperature acclimation. Consistently, Liu and Li (2005) pointed out that increases in f_H can be caused by the shortening

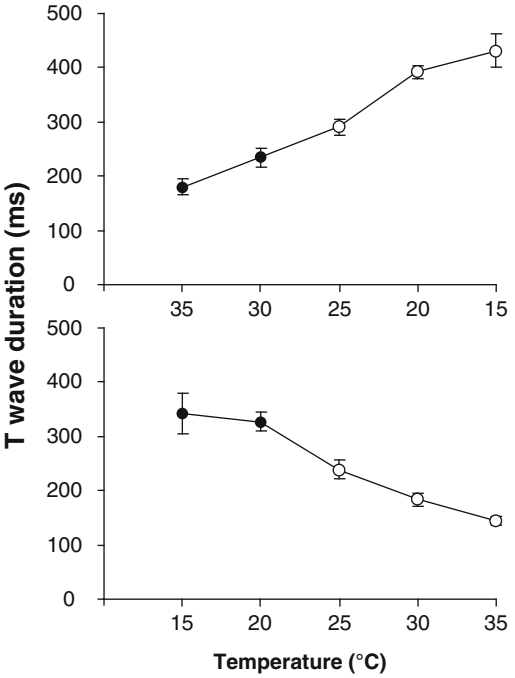

Fig. 9 Effects of temperature on the *T* wave duration for pacu, *Piaractus mesopotamicus* acclimated to 35°C (*upper panel*) and 15°C (*lower panel*). *Open symbols* indicate a significant difference ($p < 0.05$) in relation to the initial values. (from Aguiar et al. 2002)

of rest period and acceleration of atria-ventricular conduction, with a shortened rest period as the most important factor.

The Q–T interval represents the time for both ventricular depolarization and repolarization, and, therefore, roughly estimates the duration of an average ventricular action potential (VAP_D). This interval depends on heart rate. At high f_H, the duration of ventricular action potentials shorten, which decreases the Q–T interval, while the opposite occurs at low f_H (Klabunde 2004). In the vertebrate heart, VAP_D depends on body temperature, in a way that reminds of the QRS duration, i.e., it is longer at low body temperatures and shorter as temperature is increased (Shattock and Bers 1987). The conduction velocity determines the excitation and relaxation of the ventricle and, as it slows down, the ventricular activity lasts longer.

Shortened VAP_D due to increases in temperature were earlier reported for flounder ventricle by Lennard and Huddart (1991) and by Chapovetsky and Katz (2003) working on toads. The same effect was reported for the tropical species *O. niloticus* (Maricondi-Massari et al. 1998) and *P. mesopotamicus* (Aguiar et al. 2002) acclimated to 15°C and submitted to increases of temperature up to 35°C. The opposite occurred when fish acclimated to 35° were submitted to stepwise decreases in temperature down to 15°C (Fig. 11). However, the VAP_D of *P. mesopotamicus* is shorter

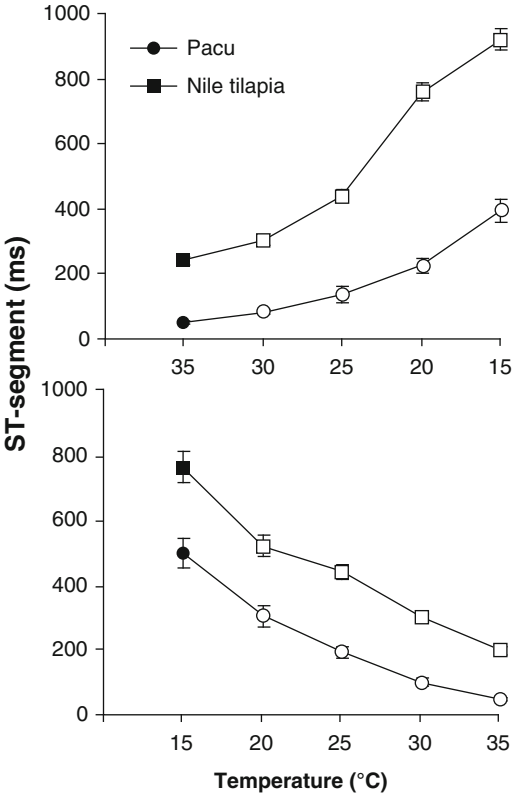

Fig. 10 Effects of temperature on the ST-segment (ms) for Nile tilapia, *Oreochromis niloticus* (Rantin and Kalinin, unpublished data), and pacu, *Piaractus mesopotamicus* (Aguiar 1996) acclimated to 35°C (*upper panel*) and 15°C (*lower panel*). *Open symbols* indicate a significant difference ($p < 0.05$) in relation to the initial values

than that of *O. niloticus* (Fig. 11), which increases f_H of *P. mesopotamicus* to values exceeding those of *O. niloticus* (Fig. 5).

Cotter and Rodnick (2006) analyzed the effects of different anesthetics on electrical properties of the rainbow trout heart, and discovered that the QRS duration and QT interval (VAP_D) were independent of f_H. According to these authors, the QT interval varies inversely with heart rate in mammals, in which cardiac output predominately increases by elevated f_H and sympathetic activation. Most fish, including rainbow trout, increase cardiac output predominately via a larger stroke volume, which depends less on electrical activity (i.e., decreasing the time from depolarization to repolarization of the ventricles would not have significant effects in stroke volume versus heart rate regulators). These authors also define how QT interval and f_H are related in unanesthetized fish, which would increase the understanding of the relationship between the electrical and mechanical activities of the ventricle.

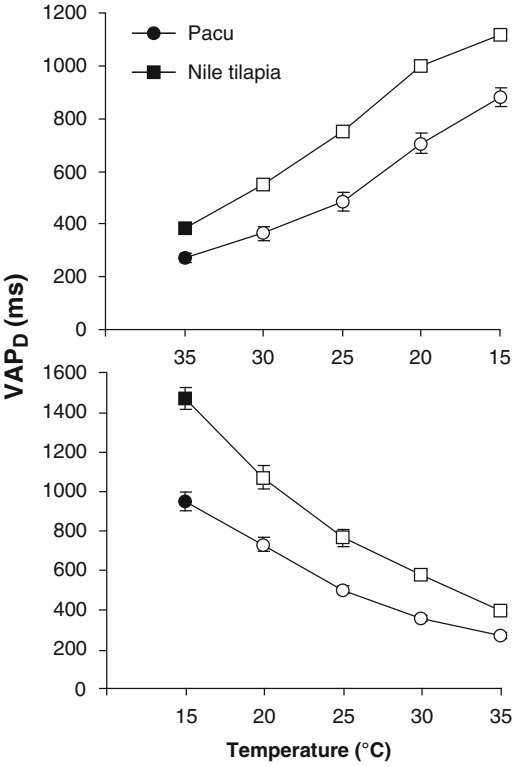

Fig. 11 Effects of temperature on the ventricular action potential duration (VAP_D–ms) for Nile tilapia, *Oreochromis niloticus* (Maricondi-Massari et al. 1998), and pacu, *Piaractus mesopotamicus* (Aguiar et al. 2002) acclimated to 35°C (*upper panel*) and 15°C (*lower panel*). *Open symbols* indicate a significant difference ($p < 0.05$) in relation to the initial values

3 In vitro Cardiac Performance

Most of the studies on cardiac contractility were developed with endothermic animals, in which cardiac muscle works in vivo on essentially constant temperatures (Hove-Madsen 1992). Furthermore, the literature dealing with the effect of temperature on cardiac Ca^{2+} management of tropical fish has been rather scarce, since most studies performed with these animals only analyzed the effect of acute changes in temperature, lacking data on the seasonal adaptive effects. In contrast, much attention has been paid to few species of temperate teleosts, specially the rainbow trout, *Oncorhynchus mykiss*, and the crucian carp, *Carassius carassius*.

Temperature modifies the rate of enzymatic reactions, including those of cardiac tissue (Marengo et al. 1997). As a consequence, cardiac contractility in fish can directly be influenced by temperature effects on the duration of active state (i.e., contraction and relaxation dynamics) (Bailey and Driedzic 1990). Altered

contraction dynamic allows the total amount of Ca^{2+} activator to bind to troponin C in a single contraction, affecting peak force. Conversely, these temperature-induced alterations in the active state can also modify contraction frequency, changing cardiac contractility with temperature (Shiels et al. 2002a).

The ability to tolerate acute temperature changes (daily and/or water column dislocation-related) is an inherent genetic property, which triggers an adequate cardiac performance to match fast thermal variations. Therefore, an adequate cardiac contractility in response to acute changes in temperatures is only present in species that possess a sufficient myocardial plasticity to avoid disturbances in electrical excitation and metabolic imbalance (Paajanen and Vornanen 2004).

As a consequence, twitch cardiac force can increase, decrease, or even remain unaltered after an acute change in temperature. For instance, ventricle strips from Nile tilapia (Costa et al. 2000), *O. niloticus*, and the frillfin goby (Rantin et al. 1998), *B. soporator*, showed a marked reduction in twitch force during acute increases in temperature from 25 to 35°C, in spite of such thermal transitions in the habitat (Fig. 11). Nonetheless, both Nile tilapia and frillfin goby presented an increase in f_H as temperature was acutely elevated (Fig. 1), which may increase cardiac output through a higher contractility, rather than by elevations in stroke volume. Additionally, chronotropy induced by high temperature has a negative inotropic effect, due to the typical negative force staircase in teleost fish (Driedzic and Gesser 1985; Vornanen 1989).

On the contrary, pacu presented an accentuated increase in chronotropism (Fig. 1) while the inotropic response remained the same (Fig. 11) during the acute elevation in temperature from 25 to 35°C (Anelli-Jr et al. 2004). A marked temperature-induced increase in cardiac output of pacu is well correlated with its high activity pattern, since the cardiac demand increases in proportion to an acute elevation in temperature.

Conversely, an acute reduction in temperature from 17 to 2°C will not significantly influence the force of contraction in the atrial tissue of salmon or trout (Fløysand and Helle 1994; Ask et al. 1981), whereas a reduction of temperature from 20 to 1°C increased the force of contraction ventricle of the crucian carp by 60% (Vornanen 1989). In these fish, the reduction in bath temperature was associated with a marked increase in the time to peak force, which would let more Ca^{2+} to enter the cell during a single contraction.

This response is related to the fact that the duration of the action potential (AP) in fish hearts (around 500 ms) is already prolonged, when compared to rat hearts (less than 100 ms) (Vornanen 1989; Møller-Nielsen and Gesser 1992; Shiels et al. 2006). (Fig. 12) On the contrary, an acute increase in temperature decreases the time available for the development of contractile force, since less time is available for Ca^{2+} to reach the contractile apparatus during contraction. According to Paajanen and Vornanen (2004), the mechanism that underlies the temperature-dependent shortening of AP duration observed in cold-acclimated crucian carp ventricular myocytes during acute increases in temperature is correlated with temperature-induced changes in the voltage-dependent rectification of the background inward rectifier K^+ current.

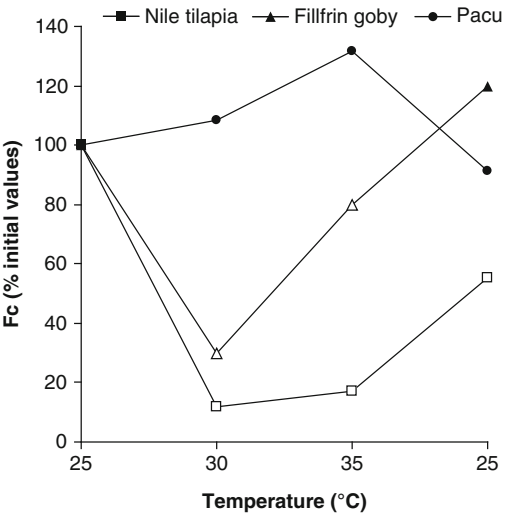

Fig. 12 Twitch force (*Fc* = % initial values) developed by ventricle strips from three species of tropical teleosts ($N = 10$ for all) acclimated to 25°C and subjected to an acute increase in temperature until 35°C and then to the subsequent return to acclimation temperature. *Open symbols* denote a significant ($p < 0.05$) difference in relation to the initial values. Results are presented as means, but error bars have been omitted for clarity. Nile tilapia from Costa et al. 2000; fillfrin goby from Rantin et al. 1998; pacu from Anelli-Jr et al. 2004

Temperature tolerance can be increased by temperature acclimation, because chronic (seasonal) temperature changes can allow the expression of myosin isoforms or Ca^{2+}-transporting proteins more adapted to the new thermal environment (Paajanen and Vornanen 2004). In addition, there is a direct correlation between these responses and the thermal history of the animal, as well as with the activity pattern of the animal at a given temperature. Therefore, fish need efficient mechanisms to achieve thermal adaptation of the cardiovascular system.

Activity levels are matched to variable temperatures, and it can be generalized that changes in cardiac function in response to acclimation is most pronounced in teleosts that can remain active at low temperatures, which contrasts with sedentary animals that become torpid or lethargic in the cold (Bailey and Driedzic 1990). On the other hand, animals that become dormant or little active in the cold may develop a non-compensatory or inverse acclimation of metabolic and enzymatic activities (Prosser 1973; Matikainen and Vornanen 1992).

Some teleosts, including rainbow trout, increase swimming performance after acclimation. An active life style at low temperatures requires compensatory changes in the function of swimming muscles and the cardiovascular system, including increased cardiac contractility and increases of myofibrillar ATPase (Aho and Vornanen 1999).

Conversely, the crucian carp becomes cold-dormant, which results in a concomitant reduction of the cardiac contraction kinetics, which results from a temperature-induced reduction in myofibrillar ATPase activity (Tiitu and Vornanen 2001). The

temperature-dependent expression of different myosin isoforms in fish hearts might be important from a ecological and physiological point of view, since slow myosin types are believed to produce force more economically than fast myosins, which would precondition cardiac muscle to a low energy supply (Tiitu and Vornanen 2001).

Vornanen (1994) described the expression of a myosin heavy chain isoform in crucian carp heart during the cold season. This isoform presents a lower myosin-ATPase activity than its summer counterpart, when an additional myosin heavy chain is expressed. This suggests that both cardiac activity and energy consumption are high in warm, normoxic summer waters but significantly reduced during cold and anoxic winter. Notably, a positive thermal compensation was absent in the ventricular myofibrils of the rainbow trout, which remains active at low temperature (Aho and Vornanen 1999).

According to Pelouch and Vornanen (1996), longer intervals between beats in cold-living fish are due to a prolonged AP duration, which can induce cardiac hypertrophy, which is a direct response to wall stretch, which expands diastolic filling. The cold acclimation involves an increase of the heart, which partly compensates adverse effects of low temperatures on cardiac contractility. These authors found that crucian carp, *C. carassius*, which had been cold-acclimated (2°C) for 3 months developed an 86% increase of ventricular mass. Activity levels are matched to variable temperatures, and it can be generalized that changes in cardiac function in response to acclimation are most pronounced in teleosts that can remain active at low temperatures, which contrasts with sedentary animals that become torpid or lethargic in the cold (Bailey and Driedzic 1990). On the other hand, animals that become dormant or little active in the cold may develop a non-compensatory or inverse acclimation of metabolic and enzymatic activities when compared to warm-acclimated animals at 22°C (Prosser 1973; Matikainen and Vornanen 1992) This cold-induced enlargement occurs mainly by an increase in collagen rather than by myofibrillar or sarcoplasmic proteins. This shifts active force production to more passive elastic elements of the extracellular matrix. Consistently, winter-acclimatized crucian carps had smaller hearts (10%) when compared to fish in the summer (Matikainen and Vornanen 1992). This is related to the inverse (or non-compensatory) temperature acclimation of the crucian carp due to its low activity during the winter. In addition, Matikainen and Vornanen (1992) described how an atypical positive force–frequency relationship in both electrically paced ventricles (Fig. 13a) or spontaneously beating hearts (Fig. 13b) from warm-acclimated crucian carp becomes more evident at higher testing temperatures.

In contrast, Shiels et al. (2002a) showed that the force–frequency response of rainbow trout heart is shifted downwards with acute increases in temperature, and upwards with acute decreases in temperature (Fig. 14). The authors state that these changes in cardiac contractility favor brief exploitation of colder niches after warm-acclimation, but not vice-versa. This response was also described in other cold-active teleosts, including the yellowfin tuna (Shiels et al. 1999) and the mackerel (Shiels and Farrell 2000).

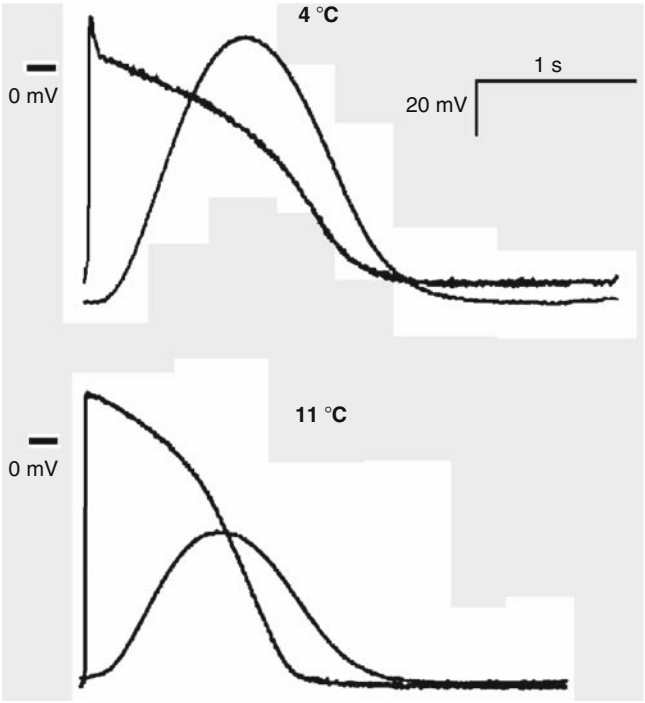

Fig. 13 Representative recordings of ventricular action potentials and associated contractions of the burbot (*Lota lota*) ventricle at 4°C and 11°C. *Scale bars* are for recordings at both temperatures. Force of contraction is in arbitrary units (from Shiels et al. 2006)

Animals subjected to similar temperature conditions and different adaptation strategies can be incorporated into behavioral patterns. Unfortunately, information on in vitro temperature acclimation is lacking with regard to cardiac function in tropical teleosts.

The species-specific differences of the inotropic responses described above are linked to the components of Ca^{2+} management in myocytes (Fig. 15). Myofilaments are activated during the E–C coupling in response to an increased cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$. can occur in response to mobilization of these ions from the sarcoplasmic reticulum (SR) as well as by influx through the sarcolemma (SL), which can occur via L-type Ca^{2+} channels and/or Na^+/Ca^{2+} exchanger (NCX) acting in the reverse mode (Hove-Madsen et al. 2000). Inversely, cardiac muscles relax when $[Ca^{2+}]_i$ is reduced back to its diastolic resting levels by Ca^{2+} transportation out of the cell via the sarcolemmal Ca^{2+}–ATPase and, in addition, mainly by the NCX and/or its accumulation inside the SR, which occurs by pumping activity via a sarcoplasmic-endoplasmic Ca^{2+}–ATPase (SERCA).

Fish myocytes have several anatomic peculiarities (Fig. 16), including a smaller myocyte diameter than in mammals, and they have peripherally arranged myofibrils and an absence of T-tubules (Farrell and Jones 1992). This results in differences

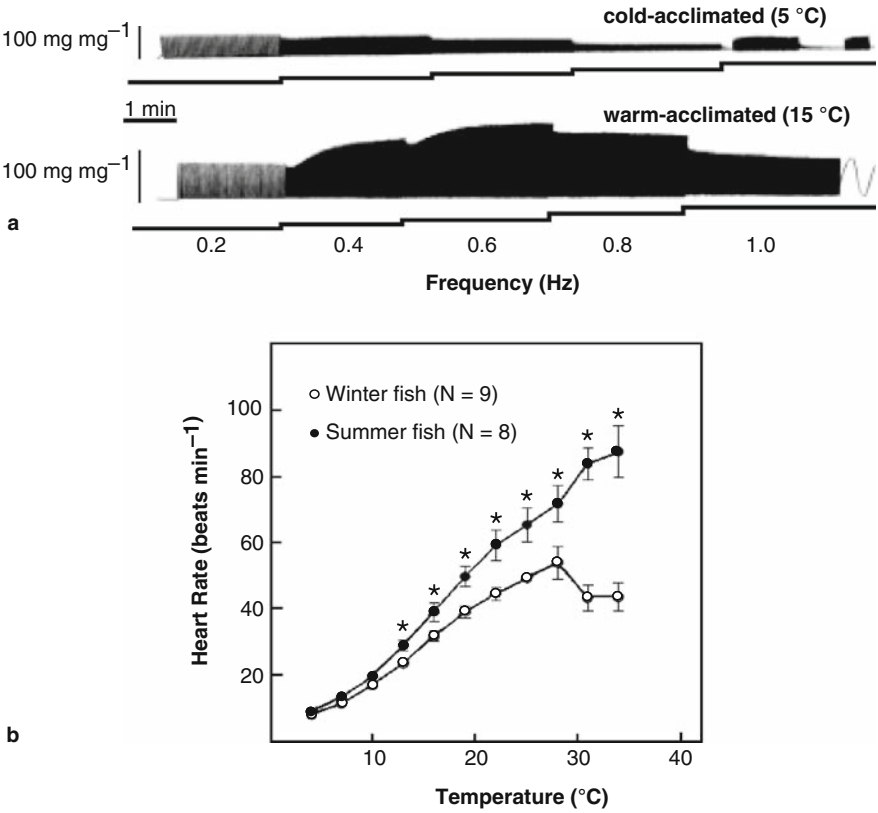

Fig. 14 **a** Original polygraph recordings of the force-frequency relationship for ventricles of warm- and cold-acclimated crucian carp during summer. Pacing rate was raised from 0.2 to 1.0 Hz at 0.2 Hz intervals. The *vertical bar* indicates 100 mg force per mg tissue wet mass. The temperature of the tissue bath was 15°C. **b** Temperature dependence of heart rate in isolated spontaneous beating crucian carp during summer and winter. Results are means ± SE. *Asterisks* indicate a value significantly different from that for winter fish (modified from Matikainen and Vornanen 1992)

in the E–C coupling, which makes the myocytes of most fish more dependent on extracellular Ca^{2+} rather than on SR Ca^{2+} stores. These conditions present a proportionally reduced contribution to Ca^{2+} management, if compared to the sophisticated regulatory mechanisms of mammals. The time and the amount of Ca^{2+} cycled on a beat-to-beat base depend directly on the activity of the Ca^{2+}-transporting mechanisms, which varies between species and depends on temperature (Gwathmey and Morgan 1991; Vornanen 1998). In fact, several physiological differences exist, and among these Ca^{2+}-transporting mechanisms and their thermal dependence will ultimately determine the pattern of the inotropic temperature responses of each species.

In fish myocytes, the temperature dependency of the NCX is lower than the value in amphibians, which itself is inferior to mammalian values (Fig. 17). This relative

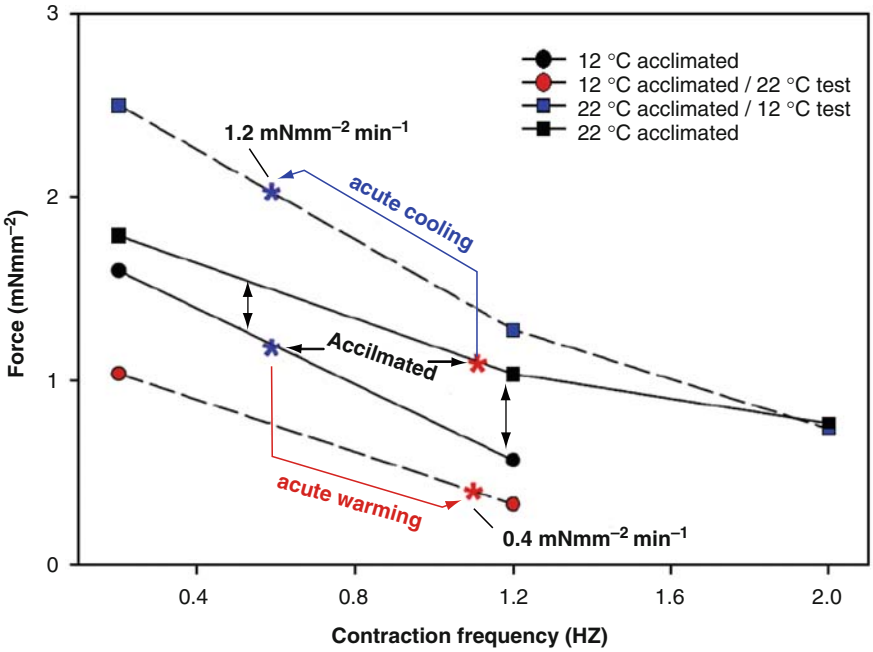

Fig. 15 The effect of acute temperature change and temperature acclimation on isometric force development from ventricular muscle from rainbow trout at high and low pacing frequencies for 12°C-acclimated fish, or high, medium and low pacing frequencies for 22°C-acclimated fish. *Asterisks* indicate the approximate in vivo heart rate under each condition. *Double-headed arrows* indicate the changes in the force–frequency relationship and in the in vivo heart rate associated with temperature acclimation. *Single-headed arrows* indicate the change in force and heart rate associated with acute increases (*red arrows*) and decreases (*blue arrows*) in temperature. Numeric values indicate the pumping capacity of the preparation (product of force and frequency) after heart rate and contractile force adjustment following an acute temperature change. (From Shiels et al. 2002)

temperature-insensitivity of NCX reflects differences in the primary structure of its proteins, which constitutes an adaptation to allow activity of the fish during exposure to low temperatures (Elias et al. 2001).

Nevertheless, it is not clear which is the advantage of temperature insensitivity to species that normally face acute and/or chronic transitions to high temperatures, considering that an increase in NCX activity in response to high temperature would allow a faster contraction–relaxation cycle. In this context, Bailey and Driedzic (1990) emphasize that ATP-dependent Ca^{2+} efflux from the cell is temperature-dependent, increasing directly with temperature.

Ca^{2+} currents through L-channels of mammals present a high temperature dependency ($Q_{10}\sim3$). For temperate fish, however, from evolutionary selection in this direction, one would expect a reduced temperature dependency of these channels (Kim et al. 2000; Shiels et al. 2000). Otherwise their cardiac contractility would be compromised after an acute decrease in temperature (Thomas et al. 1996). In

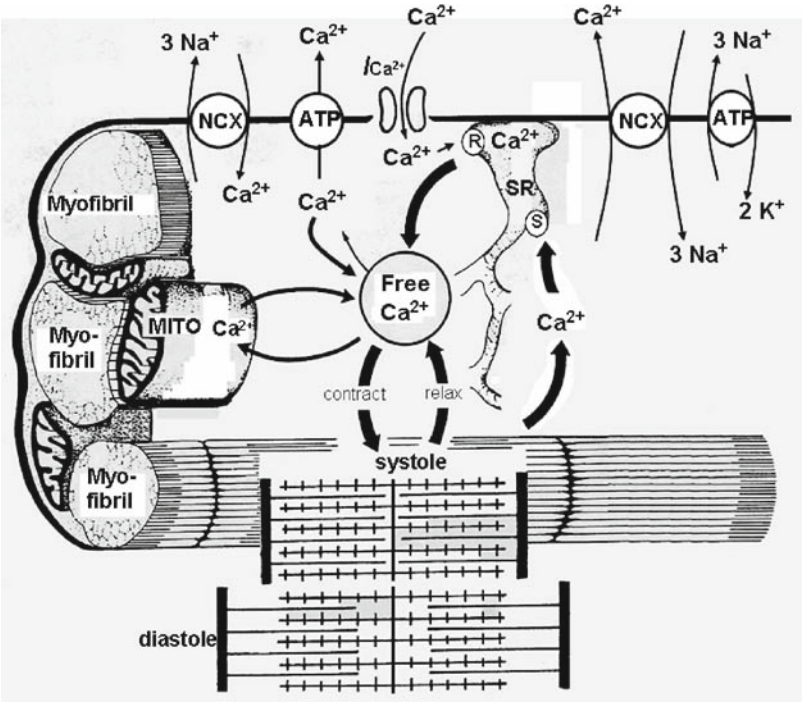

Fig. 16 NCX–Na^+/Ca^{2+} exchanger; *SR* sarcoplasmic reticulum; *R* ryanodine channel; *S* SERCA (sarco-endoplasmic reticulum calcium ATPase); *MITO* mitochondria. ICa^{2+} — (adapted from Opie 1998, for fish myocyte model)

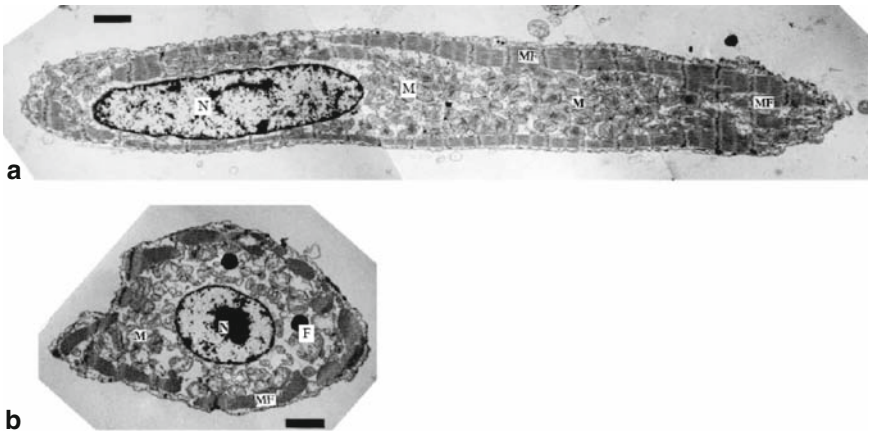

Fig. 17 Electron miographs of enzymatically isolated ventricular myocytes of rainbow trout heart. **a** A longitudinal section through a small part of a ventricular yocyte. **b** A cross-section of a ventricular myocyte. *M* mitochondria; *MF* myofibrils; *N* nucleus; *F* fat. Scale bars: 2μ M. (from Vornanen 1998)

the same direction, the high temperature sensitivity of L-channels would assure a temperature-dependent increase on the Ca^{2+} activator, which would increasse stroke volume in response to higher temperatures.

In this context, studies on teleost hearts established that Ca^{2+} influx through sarcolemma remains almost constant during changes in temperature, despite the temperature sensitivity ($Q_{10} \sim 2$) of L-channels (Kim et al. 2000; Shiels et al. 2000).

Thermal acclimation does not change Ca^{2+} current density (I_{Ca}) through L channels in the ventricular myocytes of rainbow trout and crucian carp (Vornanen 1998). The inactivation kinetics of I_{Ca} is, however, accelerated after acclimation to cold in trout, which would result in a smaller Ca^{2+} influx to the heart of cold-acclimated than of warm-acclimated trout. According to Vornanen (1998), the reduced peak amplitude of I_{Ca} could be compensated by the slower inactivation of I_{Ca}, due to the concomitant prolongation of action potential duration by low body temperature.

Furthermore, there are species-specific differences in relation to the participation of SR Ca^{2+} stores on Ca^{2+} management in fish hearts, and also between the atrium and the ventricle of the same species (atrium > ventricle) (Aho and Vornanen 1999). In spite of this, the following generalization can be made about the effects of temperature on the SR participation in the contraction–relaxation cycle: its role increases after acute elevations in temperature as well as after acclimation to cold (Shiels et al. 2002b).

A cold-induced opening of the SR Ca^{2+}-release channel renders the SR ineffective in sequestering Ca^{2+} (Tibbits et al. 1991, 1992a; Hove-Madsen 1992; Møller-Nielsen and Gesser 1992). Additionally, in the trout heart, the SERCA is temperature-dependent ($Q_{10} \sim 1.6$), and makes Ca^{2+} accumulation inside this organelle less efficient with acute reduction of temperature (Aho and Vornanen 1998; Hove-Madsen et al. 1998; Keen et al. 1994). In spite of this, the fish cardiac SR may accumulate significant amounts of Ca^{2+} during the long-lasting depolarization (Aho and Vornanen 1998).

The combination between temperature sensitivity of the SR Ca^{2+}-release channels and the temperature dependency of SERCA can minimize the role of the SR as a source of Ca^{2+} activator in hearts of fish exposed to acute fluctuations to low temperatures, as rainbow trout (Shiels et al. 2002b).

The high temperature dependency of SR Ca^{2+}-release channels in the fish heart was established by Keen et al. (1994) for rainbow trout, in which ryanodine (an alkaloid that blocks SR function) induced a twitch force decrease, which only appeared at high and unphysiological temperatures (above 15°C). More recent studies (Hove-Madsen et al. 1998, 2001) have verified that L-current magnitude itself can not fully activate myofilaments also at physiological temperatures, which implies that the SR of trout myocytes also plays some role under in vivo conditions. Additionally, Tiitu and Vornanen (2002a) observed that the SR in the atrium and the ventricle of the stenothermic and cold-active burbot, *Lota lota*, is extremely sensitive to ryanodine, even at temperatures as low as 1°C.

Different from the examples cited above, in most temperate fish ryanodine is unable to reduce the cardiac peak force at physiological temperatures. According to Thomas et al. (1996), this lack of effect of ryanodine is due to lower testing

temperatures. Atrial and ventricle strips from skipjack tuna, *K. pelamis* (Keen et al. 1992; Tibbits 1996), and from the mackerel, *Scomber japonicus* (Shiels and Farrell 2000) significantly reduced twitch force after blockade of SR Ca^{2+}-release channels by ryanodine only at temperatures above 20°C, but not at lower temperatures.

In this way, an acute transition to a high temperature can indirectly reveal an increase of SR participation in Ca^{2+} cycling in animals in which this organelle is potentially functional. Shiels and Farrell (1997) state that an acute transition to a high temperature amplifies the response to ryanodine in a more consistent manner than thermal acclimation. This implies that an increase in SR participation on Ca^{2+} management at these conditions represents an adaptive response to an acute and abrupt increase in temperature. Conversely, this implies that ryanodine channels did not suffer any evolutionary selection that could work more adequately after acute transitions to low temperatures.

Nevertheless, thermal acclimation may alter the E–C coupling process of the fish heart, given that cold-acclimation alters the relative importance of the SR Ca^{2+} cycling according to the activity pattern of the fish. The cardiac E–C coupling in the cold-dormant crucian carp is exclusively based on extracellular Ca^{2+} sources (Tiitu and Vornanen 2001), and several studies confirmed that SR Ca^{2+}-release channels from active teleosts remain functional in the cold (Shiels et al. 2002b). Indeed, morphometric analysis of the heart of perch, *Perca fluviatilis*, suggest that the SR is better developed in cold-acclimated than in warm-acclimated fish (Bowler and Tirri 1990). Consistently, Keen et al. (1994) observed a small, but significantly increased anatomical development of the cardiac SR of rainbow trout after thermal acclimation to cold. As a specific feature, this organelle increased force development only when cold-acclimated fish were subjected to acute transitions to high temperature, in particular at subphysiological stimulation frequencies. In cold-acclimated trout, this transition resulted in a more accentuated increase in twitch force, when compared to the warm-acclimated fish. Nevertheless, a high structural development of the SR was not sufficient to ameliorate force development when animals were tested at lower temperatures (Fig. 18).

Functional studies in thermally acclimated trout prove that contraction is more sensitive to ryanodine inhibition in cold-acclimated than in warm-acclimated animals, even at physiological body temperatures (∼4°C) and at physiological pacing rates (∼0.6 Hz). For instance, Aho and Vornanen (1999) recorded that the SR accelerates the recovery of contractility from inactivation and reported that the atrium and the ventricle of cold-acclimated trout allow relatively high cardiac frequencies and permit adequate cardiac outputs at low environmental temperatures. Additionally, they proved that the atrial SR contributes directly to the cytosolic Ca^{2+} under the same conditions.

There are marked species-specific differences among teleosts in relation to the SR Ca^{2+} uptake by SERCA. In this context, Aho and Vornanen (1998) reported that the cold-acclimated heart (4°C) increased the Ca^{2+} uptake rate by the SR of trout heart (complete thermal compensation).The SR Ca^{2+} uptake rate, however, indicated that rainbow trout and perch, which are adapted to low temperature,

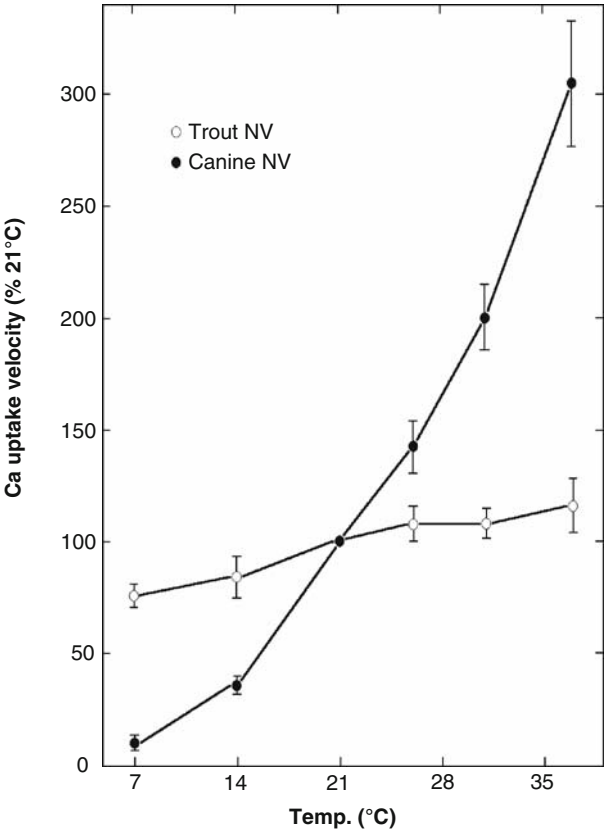

Fig. 18 Initial rates of Na^+-dependent Ca^{2+} uptake in native sarcolemmal vesicles (NV) as a function of temperature, normalized for the rate at 21°C. Extracellular Ca^{2+} concentration ($[Ca^{2+}]_0$) was 20 μM, $n = 4$ different preparations for each group, pH 7.0, and the reaction time was 2 s. NV were maintained at 4°C intil the reaction was initiated. 100% equals 3.0 and 3.5 nmlol mg, protein^{-1} s^{-1} in trot and canine NV respectively. (From Tibbits et al. 1992b)

have an increased volume and Ca^{2+}-handling efficiency of the SR (Bowler and Tirri 1990; Keen et al. 1994). These mechanisms partially compensate for the detrimental effects of cold on cardiac contractility (Fig. 19). Against this background Landeira-Fernandez et al. (2004) described that tuna species in cold waters such as albacore tunas, *Thunnus alalunga*, have an increased SR Ca^{2+} uptake capacity when compared to tropical tunas, such as yellowfin tuna, *Thunnus albacares*. Conversely, species that become inactive or dormant in the cold (e.g., crucian carp) have a limited Ca^{2+}-handling capacity, which was depressed after acclimation to low ambient temperature.

A physiological strategy to modulate the effects of changes in temperature on cardiac contractility is β-adrenergic modulation, which enhances both Ca^{2+} delivery and its subsequent removal during the contraction–relaxation cycle (Shiels et al. 2002a). Ask et al. (1981) and Keen et al. (1993) established that an increase in

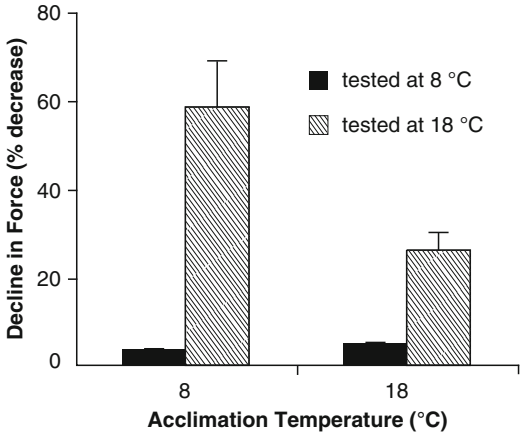

Fig. 19 Effect of ryanodine application (10 μM) on tension development in ventricular strips from rainbow trout (*O. mykiss*) acclimated to either 8 or 18°C and tested at 8 C (*solid*) or 18°C (*diagonal lines*) under the stimulation frequency of 0.2 Hz. Histograms are mean ± SEM (modified from Keen et al. 1994)

the density of β-adrenoceptors in the sarcolemma of cold-acclimated trout myocytes increases the sensitivity of trout heart to adrenaline. An upregulation of the sympathetic system increases not only the heart rate, but also contractile force (Graham and Farrell 1989). These results contrast with data for the stenothermal African catfish *Claris gariepinus*, in which myocardial β-adrenoceptor density did not change in response to temperature acclimation (15, 22, and 32°C). This may indicate that a β-adrenoceptor plasticity similar to that exhibited by eurithermal temperate teleosts is superfluous in tropical fish (Hanson et al. 2005).

In several temperate teleosts, adrenergic sensitivity is also enhanced in response to an acute reduction of temperature (Ask et al. 1981; Ask 1983; Shiels and Farrell 1997, 2000; Aho and Vornanen 2001). In the tropical teleosts *O. niloticus* (Costa et al. 2000) and *P. mesopotamicus* (Anelli-Jr et al. 2004), the effects of an acute change in temperature are opposite to those described for temperate fish, since adrenergic responsiveness of multicellular ventricle preparations increased as temperature was acutely elevated, whereas this failed to occur in *B. soporator* (Rantin et al. 1998). These species-specific differences in adrenergic responsiveness of hearts from tropical teleosts to acute elevations in temperature must be related to the fact that *B. soporator* presented an extremely pronounced temperature-induced increase in chronotropism that may assure adequate increases in cardiac output. Adrenergic modulation seems to represent an important strategy utilized by fish to increase cardiac performance when subjected to acute alterations to environmentally relevant temperatures.

To sum up the considerations, it can be stated that, in less active temperate teleosts, the SR plays a minimal role (if any) as a calcium source to the activation of myofibrils (Tiitu and Vornanen 2001), since ryanodine channels remain in an "open state" over a longer period of time, provided that the test temperatures are

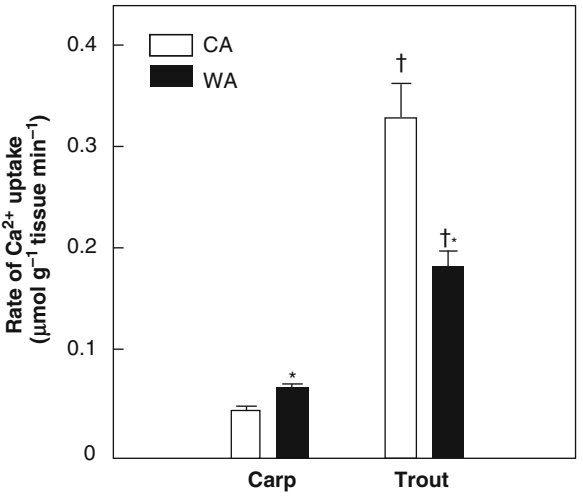

Fig. 20 Sarcoplasmic reticulum Ca^{2+} uptake rate of crude cardiac homogenates from crucian carp and rainbow trout. The results are means + SEM of 6–10 preparations. *Asterisks* indicate a statistically significant difference ($p < 0.05$) between acclimation groups. *Daggers* indicate a statistically significant difference ($p < 0.05$) between trout and carp. *CA* cold-acclimated (4°C); *WA* warm-acclimated (carp: 24°C; trout: 17°C). Modified from Aho and Vornanen 1998

low (Hove-Madsen et al. 2001). The myocytes of cold-adapted fish develop adaptive mechanisms that lead to a relative temperature insensitivity of sarcolemmal Ca^{2+}-transporting systems (particularly L-channels and NCX), which maximizes the proportional contribution of transarcolemmal Ca^{2+} fluxes to the relaxation–contraction cycle. These conditions, combined with an increase in AP duration at cold temperatures, allow a prolonged Ca^{2+} influx through the same number of Ca^{2+} channels and/or NCX molecules, which also makes sarcolemmal Ca^{2+} influx relatively independent of ambient temperature at more realistic (whole animal) situations. As a consequence, Ca^{2+} can be delivered to myosin at a rate and magnitude compatible with the low heart rates observed in most of the temperate fish (Farrell and Jones 1992; Driedzic and Gesser 1994; Lillywhite et al. 1999). This assures their survival at temperatures considered cardioplegic to an endotherm, even in species that lack a functional SR. Indeed, to active species cold acclimation is associated with an increased Ca^{2+}-handling capacity of the SR. This may allow partial compensation for the detrimental effects of cold on cardiac contractility, while an inversal thermal compensation has been described in fish that are inactive during winter (Aho and Vornanen 1998) (Fig. 20).

Conversely, two very active neotropical teleosts, the curimbata, *Prochilodus lineatus* (Kalinin, unpublished data), and the pacu, *Piaractus mesopotamicus* (Anelli-Jr et al. 2004), and two sedentary neotropical species trahira, *Hoplias malabaricus* (Olle 2003), and marbled swamp eel *S. marmoratus* (Rocha et al. 2007a,b), had a direct contribution from the SR to ventricular inotropism at physiological temperatures. This was established in fish acclimated to 25°C, using the inhibitory

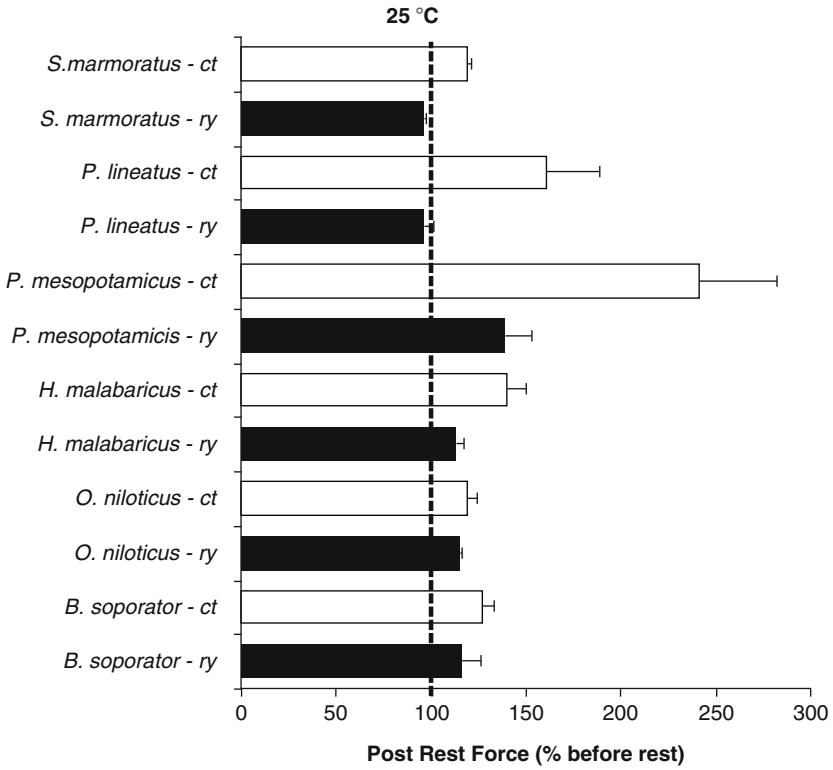

Fig. 21 Twitch force developed after a prolonged diastolic pause (5 min) in ventricle strips of fish before (*ct* = control) and after addition of 10 μM of ryanodine (*ry*)

effect of ryanodine after a prolonged diastolic pause (5 min) (Fig. 21). These data would allow us to state that tropical fish present an increased metabolic rate associated with a faster contraction–relaxation cycle, due to a shorter AP duration and a more direct participation of the SR in the E–C coupling to reduce the diffusion distances and/or faster Ca^{2+}-transporting proteins in a predictable manner. In particular, Fig. 21 shows that ryanodine had no effect on post-rest force of ventricle strips from other tropical teleosts (*O. niloticus* and *B. soporator*), irrespective of their activity pattern. This implies that in tropical teleosts, the SR participation on cardiac Ca^{2+} management is not necessarily dependent upon the activity pattern of the species. In active tropical teleosts lacking a functional SR, the transarcolemmal Ca^{2+} fluxes (mainly via L-channels and NCX) may suffice to provide an adequate myofilament activation, given that temperature-induced increases in heart rate may be enough to increase cardiac performance in response to a temperature-induced increased cardiac demand.

Interestingly, Rivaroli et al. (2006) defend the hypothesis that the species-specific differences in the dependence of the SR among tropical teleosts could be related to their phylogenetical position. While most of the tropical fish presenting a potentially

functional SR (curimbata, trahira, and pacu) belong to the superorder Ostariophysi, most tropical ryanodine-insensitive teleosts (e.g., Nile tilapia, and frillfin goby) belong to the superorder Acanthopterygii. Among the species presented in Fig. 21, however, an exception to this rule is the acantopterygian marbled swamp eel (*S. marmoratus*), in which the SR plays a functional role for the ventricular Ca^{2+} even at physiological f_H and temperatures (25 and 35°C), despite also being a highly sedentary animal (Rocha et al. 2007b). The effects of thermal acclimation to seasonally relevant temperatures are necessary to evaluate and compare the adaptive strategies developed by these animals involving a large number of subtropical and tropical teleost fish.

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