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Review

Limits to exhaustive exercise in fish☆

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Abstract

Exercise to exhaustion leads to severe metabolic, acid–base and ionic changes in fish. It has been shown that several abiotic and biotic factors can limit burst exercise performance and the recovery process in fish. This article reviews the importance of body size, temperature, fasting/starvation and training on the ability of fish to perform and recover from exhaustive exercise. It is concluded that the constraints placed on a fish prior to and following exercise reflects the large intra-specific variability in the physiological response to exercise in fish. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

Animals show many locomotor strategies which allow them to survive in various ecological niches. Many factors, such as predator–prey interactions, reproductive behaviour and habitat distributions are of profound ecological importance that depend heavily on an animal's capacity for movement (Baker, 1978). Some animals move slowly, others move extremely fast whereas others can reach high velocities but show very little stamina (Bennett, 1991). Thus, both the endurance and burst activity capacities of an animal are important determinants of many life-history characteristics. Because exercise plays an important role in the lives of most animals, quantifying the exercise capacities of various animal taxa has become a very popular research area for physiologists, behaviourists and ecologists (Bennett, 1991; Wood, 1991).

Among lower vertebrates, fish are probably the best studied group of organisms with respect to exercise physiology. The study of exercise in fishes began about 40–50 years ago with the pioneering work of Black and Brett (see Black et al., 1962, 1966; Wood, 1991; Brett 1995), which demonstrated that fish have a great capacity for both aerobic, sustained swimming and anaerobic, burst type swimming (Beamish, 1978; Wood, 1991; Moyes and West, 1995; Milligan, 1996). In particular, Edgar Black and his colleagues showed that fish possess a large anaerobic capacity, and that the post-exercise recovery process is much slower in fish compared to mammalian species.

Black's research has influenced many fish physiologists, and has helped to define several avenues for research. The first avenue of research centred around describing the physiological response to exhaustive exercise. These studies, which span

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various fish groups, focused on the controversial mechanisms of lactate removal and glycogen resynthesis (Pagnotta and Milligan, 1991; Wood, 1991; Milligan and Girard, 1993; Wang et al., 1994b; Moyes and West, 1995; Milligan, 1996; Milligan et al., 2000). Over the past decade researchers have examined closely the roles of muscle pH (Walsh and Milligan, 1989) and hormones (e.g. catecholamines and corticosteroids; Milligan, 1996) on these processes. More recently, the roles of both fatty acid (Milligan and Girard, 1993; Wang et al. 1994b) and protein metabolism (Milligan, 1997) on the post-exercise lactate patterns are being explored. The second avenue of research has focused on inter-species differences/variation in the physiological response to exhaustive exercise. Most of this research has compared the exercise capacities of flatfish and other inactive species with high performance fish, such as tuna and salmonids (Milligan and Wood, 1987a,b,c; Wood, 1991; Nelson et al. 1994; Kieffer et al. 1996). This research has provided important insights about the ecological differences/requirements between species with respect to exercise performance. The last major area, in which this review mainly focuses on, is the intraspecific (within species) variability associated with exhaustive exercise in fish. Over the past decade, researchers have shown that substantial intraspecific variation exists in the exercise physiology of various fish species (Nelson, 1990; Ferguson et al., 1993; Kieffer et al., 1994; Kieffer, 1995; Milligan, 1996). These findings have enabled researchers to identify the factors, which may place limits on exercise performance of fish, which may account for some previously documented differences in the physiological response to exercise between studies using the same experimental animal (Dalla Via et al., 1989; Kieffer et al., 1994; Kieffer, 1995). In particular, the role of body size, temperature, training, diet/nutrition, and water quality have been shown to limit both the exercise performance and recovery from exhaustive exercise.

The goals of this review are several fold: (I) to briefly describe the physiological response to exhaustive exercise in fish; (II) to describe the intraspecific variation of the physiological response in fish; (III) to describe how various exogenous and endogenous factors account for the intraspecies variability in the physiological response to exhaustive exercise and the recovery from exhaustive exercise. In particular, the role these factors play on limiting burst exercise performance in fish will be discussed. Lastly, I hope to provide future directions in the field of exercise physiology in fish.

2. Physiological response to exhaustive exercise in fish

Swimming activity in fish is normally categorized as either aerobic or anaerobic (Beamish, 1978). During sustained exercise, muscle metabolism is largely aerobic and is supported by the well-perfused red musculature (Beamish, 1978). While much of the swimming activity in fish is aerobic in nature (Beamish, 1978), periods when the capacity for this type of swimming is exceeded exist. During these events (e.g. predator–prey interactions and spawning migrations; Beamish, 1978), burst-type exercise is largely supported by anaerobic glycolysis within the white muscle (Black et al., 1966; Milligan and Wood, 1986; Wood, 1991; Moyes and West, 1995; Milligan, 1996). Unlike aerobic-type swimming, this type of exercise can be maintained for only short periods and ends in fatigue (Beamish, 1978; Wood, 1991). Given that the physiological response to exhaustive exercise in fish is well-known, and several excellent reviews have been written within the last few years (Wood, 1991; Moyes and West, 1995; Milligan, 1996), I will only summarize the general response.

As in many animals (Reptiles and amphibians: Gleeson, 1996; mammals: Hoppeler and Billeter, 1991), burst-type exercise in fish generally involves the use of three endogenous fuels stored within the white muscle: glycogen, ATP and phosphocreatine (PCr). In the early stages of activity (i.e. first 10–15 s), energy is derived largely from the breakdown of PCr and ATP (Dobson and Hochachka, 1987). However, glycogenolysis provides the majority of ATP required to sustain muscular exertion (Dobson and Hochachka, 1987; Wood, 1991; Milligan, 1996). Associated with the glycogen breakdown is an accumulation of lactic acid, which quickly dissociates into lactate and metabolic protons (Wood, 1991; Wang et al., 1994b; Kieffer, 1995; Milligan, 1996), some of which, depending on the species, leaks into the blood (Kieffer et al., 1994; Wang et al., 1994b; Milligan and Wood, 1987b; Wilkie et al., 1996, 1997). The accumulation of metabolic protons

(H^+m) in the white muscle causes a rapid reduction in both the muscle and blood pH (Milligan and Wood, 1986; Wood, 1991; Kieffer et al., 1994; Wang et al., 1994b; Kieffer et al., 1995; Milligan, 1996). Exhaustive exercise stress also causes a large disturbance of ionic, osmotic and fluid volume homeostasis (Wang et al., 1994b).

Following the exercise bout, the recovery of PCr and ATP is generally quite rapid and usually occurs within the first 1h post-exercise (Milligan and Wood, 1986; Booth et al., 1995; Wang et al., 1994b); however, the recovery patterns for these two metabolites often differ substantially between studies and species (Milligan and Wood, 1986; Dobson and Hochachka, 1987; Schulte et al., 1992; Boutilier et al., 1993; Kieffer et al., 1994; Wang et al., 1994b). Unlike the recovery of PCr and ATP, the removal of muscle lactate and the resynthesis of glycogen is much slower, and often requires up to 12 h in some species (Wendt and Saunders, 1973; Milligan and Wood, 1986; Schwalme and Mackay, 1991; Kieffer et al., 1994; Wang et al., 1994b; Booth et al., 1995; Wilkie et al., 1997; McDonald et al., 1998).

Table 1
Maximum muscle lactate concentration (μmol/g wet tissue) following exhaustive exercise in various species of fish

Species	Muscle lactate	Reference
Adult Rainbow trout (*O. mykiss*)	41 ± 2.6	Schulte et al., 1992
	~30	Kieffer et al., 1994
Adult Atlantic salmon (*S. salar*)	~45	Wilkie et al., 1997
	~37	Booth et al., 1995[a]
	~25	Brobbel et al., 1996[a]
Adult Brook trout (*Salvelinus fontinalis*)	~33	Kieffer et al., 1996
Adult Sea Lamprey (*Petromyzon marinus*)	~25	Boutilier et al., 1993
Adult Largemouth bass (*Micropterus salmoides*)	~20	Kieffer et al., 1996
Adult Smallmouth bass (*Micropterus dolomieu*)	~18	Kieffer et al., 1995[a]
Yellow perch (*P. flavescens*)	~24	Schwalme and Mackay, 1991
Roach (*Rutilus rutilis*)	6 ± 1	Dalla Via et al., 1989
Adult Starry Flounder (*Platichthys stellatus*)	~10	Milligan and Wood, 1987b
Juvenille Winter Flounder (*Pleuronectes americans*)	~9	[b,c]
Juvenille Shortnose sturgeon (*Acipenser brevirostrum*)	~6	[d]

[a] Indicates post-angling lactate levels.
[b] Indicates whole body analysis.
[c] Frank and Kieffer, unpublished.
[d] Kieffer, Brillant and Litvak, unpublished.

Exhaustive exercise also causes an elevation in circulating levels of plasma 'stress' hormones, corticosteroids (e.g. cortisol) and catecholamines (e.g. noradrenaline and adrenaline; for reviews, Gamperl et al., 1994; Milligan, 1996). In brief, the levels of noradrenaline and adrenaline are highest immediately after exercise (Gamperl et al., 1994; Milligan, 1996), but cortisol does not peak until about 1–2 h post-exercise (Gamperl et al., 1994). The release of catecholamines generally has a wide array of effects including the facilitation of oxygen delivery to the tissues (Tang et al., 1989) and the mobilization of energy stores. The main effects of corticosteroid release are in the mobilization of energy reserves (Gamperl et al., 1994), however, cortisol has recently been implicated in the post-exercise recovery process (Milligan, 1996).

Overall, the process of recovery from burst-type exhaustive exercise in fish has been well studied, and many mechanisms involved have been described (Wang et al., 1994b). Moreover, numerous interspecific (i.e. between species) differences in these processes have been shown (Milligan and Wood, 1987b,c; Dalla Via et al., 1989; Nelson, 1990; Milligan et al., 1991; Pagnotta and Milligan, 1991; Boutilier et al., 1993; Kieffer et al., 1996; i.e. maximum lactate accumulation, see Table 1). It is also evident from previous studies that there is considerable variability in the physiological responses to exhaustive exercise within any given species (i.e. intra-specific variability; e.g. Dalla Via et al., 1989; Ferguson et al., 1993; Kieffer et al., 1994; Nelson et al., 1994; see Milligan 1996, for summary figures). In rainbow trout, for example, post-exercise lactate values often vary substantially between studies (Wieser et al., 1985; Dobson and Hochachka, 1987; Schulte et al., 1992; Fergu-

son et al., 1993; Krumschnabel and Lackner, 1993; Milligan and Girard, 1993; Kieffer et al., 1994; Wang et al., 1994b), as do the resting levels of various muscle energy metabolites (Dobson and Hochachka, 1987; Ferguson et al., 1993; Milligan and Girard, 1993; Kieffer et al., 1994; Wang et al., 1994b; see Wang et al., 1994a, for an excellent review of the literature). Similarly, the time needed for these physiological variables to recover after exhaustive exercise often varies between studies. For instance, recovery of muscle lactate may take about 6–8 h in rainbow trout (Milligan and Wood, 1986; Scarabello et al., 1991; Milligan and Girard, 1993; Kieffer et al., 1994). The time required for muscle glycogen resynthesis following exhaustive exercise can also vary from about 4–6 h (Scarabello et al., 1991; Schulte et al., 1992; Milligan and Girard, 1993) to as much as 12 h (Milligan and Wood, 1986). Finally, the recovery patterns of certain blood variables (e.g. lactate and blood metabolic protons) may also differ substantially between studies using rainbow trout (Milligan and Wood, 1986; Milligan and Girard, 1993; Kieffer et al., 1994). Thus, the physiological variability following exercise can be as great within a species as it is between species.

The issue of intraspecific variability as it relates to exhaustive exercise studies has been recognized by most researchers in this field, but only recently has it been addressed in a quantitative manner. Earlier literature suggests that the intraspecific variation in the physiological response to exercise could have been the result of differences in exercise protocols and/or the protocols used to acquire blood and tissue samples (Tang and Boutilier, 1991; Wang et al. 1994a; Kieffer, 1995). However, more recently researchers have shown that several factors may contribute to this intraspecific variability, but their relative importance is often difficult to ascertain in comparisons between different studies. This review focuses on some of these factors and describes how they limit exhaustive exercise in fish.

3. Limits to exhaustive exercise

During burst-type swimming, many physiological and biochemical systems are rapidly turned on (Dalla Via et al., 1989; Wood, 1991; Milligan, 1996), and these systems rapidly approach their limits (i.e. metabolic fuels are depleted), which may influence exercise performance (Milligan, 1996). Because exercise involves the interactions of many body systems, it is considered an integrated measure of an animal's fitness for a particular environment (Nelson, 1989). Given that the capacity for exercise is highly variable (Kieffer, 1995; Milligan, 1996), exercise to exhaustion, therefore, can be a useful model system to study regulatory processes and allow the determination of rate limiting factors in exercise performance and recovery in fish (Kieffer, 1995; Milligan, 1996).

Before describing what factors limit exercise performance in fish (see Fig. 1), it is important to describe which processes set the limits for performance. Since white muscle energy stores are used to support burst swimming, it is hypothesized that the concentrations of white muscle energy reserves (glycogen, ATP, PCr) are critical (Moyes and West, 1995). Thus, a potential limitation to burst activity could be the lack of 'on-board' energy reserves within the muscle (Fig. 1). Limitations could also be the result of the accumulation of metabolic end-products and/or the ability of the animal to recover from exhaustive exercise (Fig. 1). A priori, one might predict that there are some selective advantages associated with rapid recovery from exhaustive exercise. For example, fast recovery of white muscle energy fuels (PCr, ATP and glycogen) following activity would permit fish to perform subsequent bouts of exercise. Lastly, limitations to exercise performance could be the result of a combination between the levels of anaerobic fuels and the time for recovery following exhaustive exercise. As some literature suggests, a metabolic tradeoff may exist between balancing the levels of anaerobic fuels with reduced post-exercise recovery times. Thus, it may be more advantageous to 'cap' the levels of stored metabolic fuels but recover these more rapidly following exercise. In other words, store enough fuels to support anaerobic metabolism, but recover them quickly so subsequent and rapid bouts of exercise are possible. These balances and tradeoffs, however, would probably have a strong ecological component, and would depend on the foraging and reproductive strategies, the life-history stage and the habitat requirements of the species.

The magnitude of the physiological response to exhaustive exercise has traditionally been assessed by monitoring the levels of white muscle energy reserves (e.g., glycogen, ATP and PCr) and accumulated end-products (e.g., lactate) prior to and following the exercise regime. As indicated above, the exercise-induced imbalances in metabolite lev-

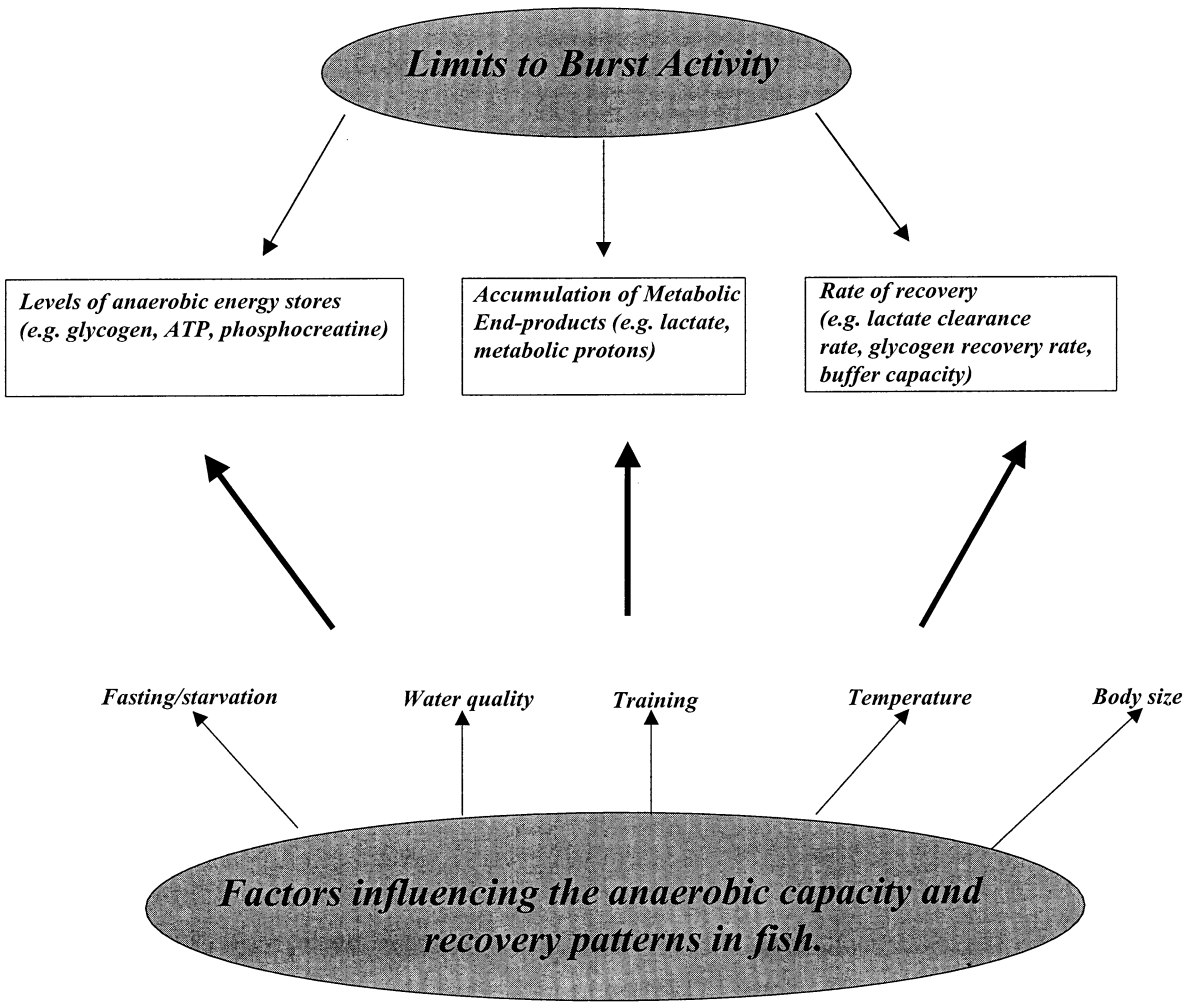

Fig. 1. Schematic representation of the limits to exhaustive exercise in fish, and the factors that influence both the anaerobic capacity and recovery patterns following exercise in fish.

els return to resting levels in minutes to several hours, depending on the metabolite (Moyes and West, 1995; Milligan, 1996). When a fish is considered recovered, therefore, depends on which metabolite is measured. Since each metabolite recovers at a different rate following exhaustive exercise, I have chosen, for illustrative purposes, to focus mainly on phosphogen, adenylate and lactate patterns. Lactate has been deemed the most useful parameter on which to base the rate of recovery of carbohydrate status (see Moyes and West, 1995, for additional details).

4. Factors that limit exercise performance and the post-exercise recovery process in fish

It has been proposed that several biological and environmental factors influence both the pre- and post-exercise condition of fish (Dalla Via et al., 1989; Pearson et al., 1990; see Fig. 1). Among other things, biological constraints can include factors such as body size, nutrition and training effects. Environmental constraints, on the other hand, can include factors such as water temperature, oxygen levels, pH levels and salinity.

4.1. Body size

Many studies have focused on the relationship between body size and aerobic metabolism (Schmidt-Nielsen, 1984). In particular, the relationship between body size and the activities of aerobic enzymes has been well studied (see Childress and Somero, 1990; Somero and Childress, 1990; Goolish, 1991, 1995, for reviews). More recently, however, researchers have focused on the influence of body size on glycolytic enzyme activity (Goolish, 1991, 1995) and the post-exercise physiology of fish (Goolish, 1989; Ferguson et al., 1993; Kieffer et al., 1996; McDonald et al., 1998). These studies show that body size places important limitations on the level of white muscle energy stores and the metabolic disturbance following brief periods of burst activity. For example all the energy stores used to support burst activity scale with body size in rainbow trout (Ferguson et al., 1993). Smaller trout have greater amounts of PCr but lower amounts of ATP and glycogen (Ferguson et al., 1993). Similar findings have been noted for two sizes of cold acclimated (5°C) rainbow trout (Kieffer and Tufts, 1998). Recently, it has been shown that O+ salmon (6–8 cm) had higher resting PCr levels compared to 1+ salmon (~ 12 cm) (McDonald et al., 1998).

Do these reduced muscle energy stores noted for small fish limit the anaerobic capacity (i.e. production of lactate) of small fish? In general, the anaerobic capacity increases with body size in salmonids (Goolish, 1989; Ferguson et al., 1993; Goolish, 1995; Kieffer et al., 1996; McDonald et al., 1998), as does the anaerobic energy expenditure (i.e. cost of burst-activity; Fig. 2; McDonald et al., 1998). The increased post-exercise metabolic disturbance in larger fish is related to a greater utilization of both ATP and glycogen stores following exercise (Ferguson et al., 1993).

The fact that smaller fish have a smaller anaerobic capacity (Ferguson et al., 1993) is also supported by a theoretical analyses of the power requirements associated with aerobic and anaerobic swimming (Goolish, 1989). These theoretical considerations reveal that smaller fish can support some energetic aspects of burst swimming through aerobic processes (Goolish, 1989). Although larger fish have a greater anaerobic capacity (Goolish, 1989; Ferguson et al., 1993; Kieffer et al., 1996), there appears to be an upper limit to this source of energy production. Several explanations exist to describe why an increase in anaerobic capacity cannot continue indefinitely with increases in body size (see Goolish, 1991, 1995, for reviews). One major consideration is that glycogen isn't stored at high levels in fish muscle (about 1% of muscle weight; Navarro and Gutierrez, 1995), and it has been suggested that the controlling factor for lactate production may be related to white muscle glycogen stores (Moyes and West, 1995). Given this limitation, a large

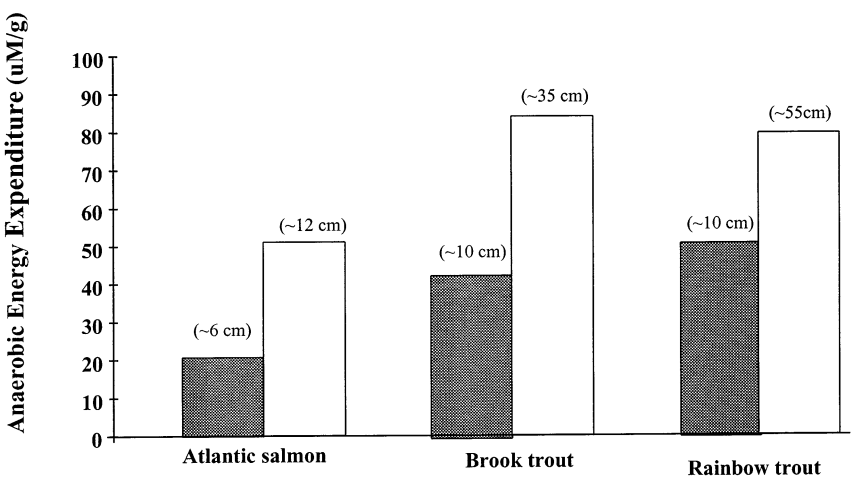

Fig. 2. Effects of body size on anaerobic energy expenditure (AEE) in Atlantic salmon (McDonald et al., 1998), Brook trout (Kieffer et al., 1996) and Rainbow trout (Ferguson et al., 1993). AEE was determined by the following equation: $AEE = (\Delta lactate \times 1.5) + \Delta ATP + \Delta PCr$, where Δ is the difference between values for resting animals and those at exhaustion (see McDonald et al., 1998, for details).

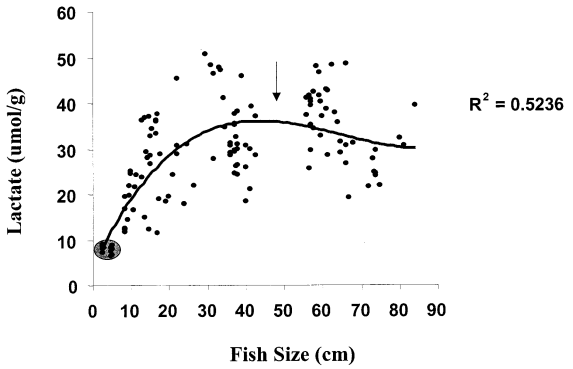

Fig. 3. The relationship between fish length and the maximum level of white muscle lactate (i.e. anaerobic capacity) in various salmonid species. The anaerobic capacity begins to decline at lengths greater than 50 cm. Data from: Rainbow trout (Ferguson et al., 1993; Kieffer et al., 1994), Brook trout (Kieffer et al., 1996, Kieffer, unpublished data), Atlantic salmon (Booth et al., 1995; Wilkie et al., 1996, 1997, Rossiter, unpublished, Galloway and Kieffer, unpublished, Kieffer and Wakefield, unpublished). The data surrounded by the shaded circle (Kieffer and Wakefield, unpublished) represent whole body lactate levels.

difference between the energy requirements at maximum speed (i.e. to overcome frictional drag) and the available energy for burst activity in extremely large individuals may exist (Goolish, 1991). In addition, the energetic costs (i.e. recovery processes) and benefits (i.e. potential for burst activity) of anaerobic metabolism should be balanced. In agreement with theoretical arguments, data from my lab and others (B. Tufts, Queen's University, Kingston, ON) partially supports an optimal size for the exploitation of anaerobic potential (i.e. capacity for burst activity) in salmonids (Goolish 1991; Fig. 3). For example, as fish size increases past a certain length (> 50 cm), the anaerobic potential plateaus or declines slightly (Fig. 3; see Goolish, 1991, for theoretical arguments). These data, therefore, indicate that metabolic tradeoffs exist between the amount of glycogen a species should store and the amount of metabolic work they are capable of. Although this interpretation is limited to salmonids, it still provides a framework for further study.

Besides muscle glycogen concentrations, anaerobic capacity may also be limited by the white muscle buffering capacity (Hochachka, 1961; Ferguson et al., 1993; Kieffer, 1995). For example, metabolic protons caused by exhaustive exercise have to be buffered, as pH has been implicated in the recovery of muscle metabolites in fish (Walsh and Milligan, 1989). However, the relationship between white muscle buffering capacity and body size is still not clear. Some researchers have found that the white muscle buffering capacity increases (Somero and Childress, 1990), decreases (Nelson and Magnuson, 1987) or does not change (Ferguson et al., 1993) with increases in body size. The lack of a relationship between body size and buffering capacity (i.e. Ferguson et al., 1993) supports the recent findings that larger fish have a greater reduction in muscle pH after exercise (Ferguson et al., 1993). Thus, it is conceivable that under certain conditions very large fish would have such a large decrease in muscle pH following exercise that metabolite recovery may be compromised. In turn, this could potentially place a maximum limit on how much glycogen a fish should store (Goolish, 1995).

As indicated above, there is now considerable evidence to suggest that body size influences and/or limits the post-exercise physiological response in fish. Most of this evidence is supported from data on active fish species (e.g. salmonids). However, the relationship between anaerobic metabolism and fish size is quite variable and can be influenced by an animal's lifestyle and locomotor habits (Somero and Childress, 1990). A more pronounced scaling relationship for glycolytic enzyme activity (e.g. lactate dehydrogenase) seems to occur in fishes that frequently display periods of burst activity (Somero and Childress, 1990) as compared to that in benthic species (Childress and Somero, 1990). As noted above, a strong positive relationship exists between body size and the post-exercise response in trout (Goolish, 1989; Ferguson et al., 1993), a species with a high capacity for both aerobic and anaerobic metabolism. In contrast, little is known about the relationship between the post-exercise response and body size in fish with fundamentally different lifestyles and foraging strategies. Recently, however, it has been shown that body size does not influence the post-exercise response in two sizes of largemouth bass (a sit-and-wait predator), but it strongly affects these variables in brook trout (an active predator) (Kieffer et al., 1996). The level of sprint performance required for an animal's predator-prey interactions therefore may be an important selective pressure determining glycolytic power (Childress and Somero, 1990; Kieffer et al., 1996). For example, because sit and wait predators attack their prey from very short distances, one might expect

that large and small individuals of the same species would not require different levels of anaerobic power (Childress and Somero, 1990). In contrast, active species must often maintain burst swimming speeds over considerable distances. Therefore, large and small individuals of a given active species (i.e. salmonids) may require different anaerobic capacities to overcome the increases in frictional drag and to maintain size-independent capacities for burst swimming (Childress and Somero, 1990; Goolish, 1991).

From the above analysis, there is a relationship between body size and the post-exercise response in some species of fish. However, the influence of body size on the post-exercise recovery process is not well known. Given that smaller fish generally experience a smaller metabolic disturbance compared to larger fish (see Figs. 2 and 3; Ferguson et al., 1993; McDonald et al., 1998), one may expect that smaller fish recover from exhaustive exercise faster compared to larger fish. To date, however, there are no studies that directly compare the post-exercise metabolite recovery process between small and large fish. Clearly, this is a good area for future research.

4.2. Temperature

Temperature is one of the most important physical factors in the environment of ectothermic organisms. Because fish are ectotherms, their body temperatures conform to the environmental temperature. Since water temperature can range from -1.5 to greater than 40°C, fish often experience large temperature changes, which may be either acute or seasonal, within their life-histories (Prosser, 1991; Taylor et al., 1997). It has been shown that survival is possible at various water temperatures because fish can modify many aspects of their physiology and biochemistry in response to temperature change (Prosser, 1991).

It is known that many physiological and biochemical processes are modified when fish are acclimated to different temperatures (Prosser, 1991; Taylor et al., 1997). At the cellular level, adaptations that occur in response to temperature changes involve changes in membrane fluidity (Hazel, 1993), enzyme concentrations (Guderley and Gawlicka, 1992), and substrate for energy production (Walesby and Johnston, 1980; Kieffer et al., 1994). Temperature has also been shown to influence cardiac output and tissue perfusion rates (Barron et al., 1987; Farrell, 1997), muscle fiber recruitment (Rome et al., 1984), and muscle contractile properties (Johnson and Johnston, 1991). Taken together, these results suggest that temperature may limit swimming performance (Taylor et al., 1997) and influences the physiological response to exhaustive exercise in fish. To date, most thermal research has focused on aerobic swimming (reviewed in Brett, 1995). This is probably the result of the effects that temperature acclimation has on aerobic pathways (e.g. enzyme changes and changes in mitochondria numbers; Prosser, 1991), which are not so apparent for the anaerobic pathway (Prosser, 1991; Hazel, 1993). Only recently has there been some emphasis on describing the influence of temperature on the physiological response to exhaustive exercise in fish (Wieser et al., 1985; Dalla Via et al., 1989; Kieffer et al., 1994; Wilkie et al., 1997); much of the research has focussed on the importance of temperature on the physiological response to 'catch and release angling' in salmon (Wilkie et al., 1996, 1997). Others have used temperature as a 'probe' to examine the effects of temperature on the strategies for acid–base regulation following exercise in salmonids (Kieffer and Tufts, 1996). Lastly, some researchers have described temperature as an important factor which may account for some variability between studies (Dalla Via et al., 1989; Kieffer et al., 1994). Whatever the rationale for the research, these studies have shown, to some degree, that temperature influences (i) the storage, utilization and/or recovery of the fuels (e.g. PCr, ATP, glycogen) required for burst exercise and (ii) the production and removal of metabolic end-products following burst activity. Therefore, temperature undoubtedly places limits on a fish's ability to perform and recover from burst-type exercise.

Given the large effects of temperature on food conversion efficiency and growth rates in fish (Jobling, 1997), it is surprising that temperature doesn't have larger effects on white muscle energy fuels. Of the energy fuels needed to support exhaustive exercise, only the levels of PCr show any trend with water temperature. Overall, acclimation to warm temperatures results in increases in the concentration of white muscle PCr of several species of fish (Walesby and Johnston, 1980; Dehn, 1992; Kieffer et al., 1994; compare Booth et al., 1995 with Wilkie et al., 1997). Levels of white muscle ATP remain independent of temper-

ature in various salmonid species (Kieffer et al., 1994; Wilkie et al., 1997; Kieffer and Tufts, 1998; Galloway and Kieffer, unpublished data). White muscle glycogen levels also remain unaffected by acclimation temperature (Kieffer et al., 1994; Kieffer and Tufts, 1998; Galloway and Kieffer, unpublished data, Kieffer and McDonald, unpublished data), although there is some variability between studies (Wilkie et al., 1996, 1997).

In agreement with many studies on salmonids, peak post-exercise white muscle lactate concentrations are not influenced by acclimation temperature (Table 2; Kieffer and McDonald, unpublished data). In addition, the overall anaerobic energy expenditure (AEE) is not affected by acclimation temperature (Table 2). AEE is an important measurement because it reflects the ATP generation/use from glycogen, ATP and PCr (McDonald et al., 1998). Since temperature does not influence the AEE, the available data show that salmonids rely on all three energy fuels equally regardless of acclimation temperature (Table 2).

With the exception of PCr, temperature influences the recovery rates of most metabolites. For example, the recovery rates for ATP and glycogen are slower at cooler temperatures (Kieffer et al., 1994; Wilkie et al., 1997; Galloway and Kieffer, unpublished data). The slower return of ATP and glycogen to resting levels in cooler water could be associated with diffusive and enzymatic limitations that negatively influence oxidative processes in the muscle (Wilkie et al., 1997). In general, acclimation to warmer temperatures increases the lactate clearance rates in fish (Q_{10} values range from 1.35–1.7; except, Wilkie et al., 1996, see Table 3). Besides metabolite recovery, there is some evidence that temperature influences the oxygen debt accumulated during intense exercise. When Nile tilapia (*Oreochromis nilotica*) were chased to exhaustion at 12, 24 and 34°C the resultant increase in oxygen uptake during recovery increased substantially with temperature, indicating that a greater oxygen debt was accumulated at higher temperatures (McKenzie et al., 1996). This may limit the duration of anaerobic burst swimming at elevated temperatures or may affect the recovery process.

As noted above, temperature places several limits on fish exercise performance. However, these effects mainly influence the recovery process, rather than on the stores of metabolic fuels. Thus, cold acclimation doesn't appear to influence metabolic work, but only the costs associated with recovery. This has important implications for a fish. For instance, under stressful situations (e.g. hypoxia, predator-prey interactions, negotiating waterfalls for migratory purposes), fish rely on anaerobic metabolism. However, if the stressful event requires subsequent, rapid burst activity or

Table 2
Changes in muscle lactate, PCr and ATP concentrations (in μmol/g wet muscle tissue) and anaerobic energy expenditure (AEE) following exhaustive exercise in fish species acclimated to different temperatures[a]

Species	T °C	ΔLac$^-$	ΔPCr	ΔATP	AEE[b]	Source
Rainbow trout (*O. mykiss*)	5	30	30	2.5	~78	Kieffer et al., 1994
	18	30	40	4.3	~89	
Atlantic salmon (adult) (*S. salar*)	12	36	29	6.6	~90	Wilkie et al., 1997
	18	34	13	8.2	~72	
	23	35	17	5.5	~75	
Atlantic salmon (juvenile) (*S. salar*)	6	40	27	7	~94	[c]
	18	41	23	6	~91	
Herring (larvae) (*Clupea harengus* L.)	5	5	15	2	~25	Franklin et al., 1996[d]
	12	6	15	2	~26	

[a] All values represent the difference between resting (control) values and those values immediately following exercise (i.e. time 0 h). all values are approximated.

[b] AEE was determined by the following equation.

$$AEE = (\Delta lac \times 1.5) + \Delta ATP + \Delta PCr$$

where Δ is the difference between values for resting animals and those at exhaustion (see McDonald et al., 1998) for details.

[c] Galloway and Kieffer, unpublished.

[d] Represents whole body measurement. ΔLac, ΔPCr, and ΔATP refer to differences between pre-exercise (control) values and immediate post-exercise in muscle lactate, PCr and ATP, respectively.

Table 3
Lactate recovery rates following exercise in various species of fish acclimated to different temperatures[a]

Species	T °C	Lactate[b] ($t = 0$ h)	Lactate[b] ($t = 4$ h)	Clearance rate[c] (μmol/g per h)	Q_{10}	Source
Rainbow trout *(O. mykiss)*	5	30	20	2.5	1.7	Kieffer *et al.*, 1994
	18	30	10	5		
Atlantic salmon *(S. salar)*	6	37	3	8.5	1.02	Wilkie *et al.*, 1996
	20	45	10	8.8		
Atlantic salmon *(S. salar)*	12	41	18.5	5.6	1.52[d]	Wilkie *et al.*, 1997
	18	39	7.5	7.9		
	23	41	5.6	8.9		
Atlantic salmon *(S. salar)* (juvenile)	6	41	15	6.5	1.35	[e]
	18	43	6	9.3		
Roach *(R. rutilis)*	4	6	1	1.3	1.65	Dalla Via *et al.*, 1989
	20	12	0.5	2.9		

[a] All values are estimates.
[b] Lactate values are expressed as μmol/g wet tissue.
[c] All rates are based on the difference between 0 and 4 h post-exercise. Note: Due to a general lack of available data, this analysis was based on two data points (i.e. 0 and 4 h); it should be noted that the temperature relationship may not be linear, as implied by the above analysis.
[d] Q_{10} measured between 23 and 12°C. $Q_{10} = (k_2/k_1)^{10/(t2-t1)}$, after Prosser (1991), where k_1 and k_2 are rates of reaction (rate constants) at temperatures t_1 and t_2, respectively.
[e] Galloway and Kieffer, unpublished data.

anaerobic metabolism, then cold acclimated fish may be partially constrained. Since the recovery patterns for PCr are not greatly affected by temperature (Kieffer et al., 1994; Booth et al., 1995; Franklin et al., 1996; Galloway and Kieffer, unpublished data), however, it should be noted that cold acclimated fish probably still have some capacity for subsequent bursts. The ecological and behavioural aspects of burst swimming have not been examined in great detail; this would be an ideal area for future research.

The above discussion focuses on fully acclimated fish. Long-term acclimation of fish to a new temperature results in many physiological and biochemical adjustments to maintain biological function (Prosser, 1991). Because the cellular changes associated with temperature acclimation are not instantaneous, one may expect that the physiological responses, and thus any limitations, may be quite different when fully acclimated fish are compared with those that have experienced an acute temperature change. To date, however, few studies have determined the relationship between acute temperature changes and whole animal response challenges such as exhaustive exercise. Unpublished data from our lab (Galloway and Kieffer, unpublished) suggests that the recovery period for glycogen and lactate is slower in fish exercised at 12°C and allowed to recover at 6°C versus fish exercised at 12°C and allowed to recover at 18°C (Fig. 4). Our results, therefore, suggest that acute temperature changes may limit exercise performance (i.e. the ability for subsequent burst activity) more greatly than in fish acclimated fully to a given temperature.

4.3. Fasting/starvation

Due to the spatial and temporal patchiness of food in nature, periods of food deprivation are common place in the lives of many fish. It is known that fish can survive for long periods without food and for many migrating species (e.g. salmonids) a fasting period forms part of a natural life cycle. In addition to the natural periods of food deprivation, it is a common practice in physiological studies to deprive animals of food prior to experimentation. In particular, many fish physiologists studying swimming metabolism often fast their animals for 3–10 days prior to experi-

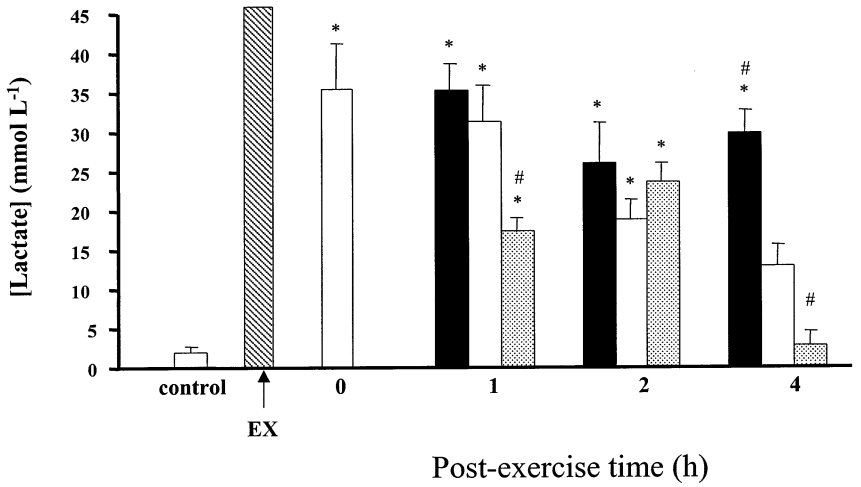

Fig. 4. Changes in the white muscle lactate concentrations of juvenile (~ 30 g) Atlantic salmon (*S. salar*) following exhaustive exercise. Fish were acclimated and exhaustively exercised (5 min. manual chasing) at 12°C (open bar). Fish were allowed to recover at either 6°C (black bar), 12°C (open bar) or 18°C (stippled bar) over a four hour period. Significant differences ($P < 0.05$) from control values are indicated by an asterisk. # represents significant difference from values of 12°C fish at each sampling period. The vertical hatched bar represents the period of exercise (EX). All data are expressed as means ± S.E.M. Unpublished data of Galloway and Kieffer.

mentation (Tang and Boutilier, 1991; Kieffer et al., 1994; Wang et al., 1994b; Kieffer and Tufts, 1998). It has been shown that starvation and short term fasting influence a number of physiological (e.g. energy reserves) and biochemical (e.g. enzyme levels) effects in fish (Sheridan and Mommsen, 1991; Vijayan and Moon, 1992; Navarro and Gutierrez, 1995), which could potentially affect swimming metabolism.

With respect to muscle energy stores, most research has focused on the effects of fasting on glycogen levels. It is well known that fasting can markedly influence the white muscle glycogen in fish (see Table 4). Recently, it has been shown that fasting fish for four or five days can cause significant reductions in the white muscle glycogen stores of juvenile trout (*Oncorhynchus mykiss*) (Scarabello et al., 1991; Kieffer and Tufts, 1998). This pattern of glycogen depletion following a fasting period is common among other species of fish, including yellow perch (*Perca fluvescens*), brown trout (*Salmo trutta*), and roach (see Table 4). It should be pointed out, however, that experimental fasting effects are likely dependent on endogenous and exogenous factors. It is known that several factors such as temperature and photoperiod, fish age/size, and reproductive state may have important influences on the experimental results (for a review, see Navarro and Gutierrez, 1995). For example, in brown trout, significant decreases in glycogen concentrations were found at eight days fasting in summer (Table 4), but only at 30 days fasting in winter (Navarro et al., 1992). Similarly, glycogen levels were significantly

Table 4
Variations in muscle glycogen levels following various period of fasting/starvation in fish

Species	Non-fasting glycogen levels	Fasting glycogen levels	Time fasted	Source
Brown trout (*S. fario*)	0.30% of muscle	0.20%	8 days	Navarro et al., 1992
Rainbow trout (*O. mykiss*)	30 μmol/g wet tissue	15 μmol/g wet tissue	7 days	Kieffer and Tufts, 1998
	20 μmol/g wet tissue	12 μmol/g wet tissue	7 days	Kieffer and Tufts, 1998
Rainbow trout	56 nmol/mg dry tissue	27 nmol/mg dry tissue	5 days	Scarabello et al., 1991
Perch (*P. fluviatilis*)	0.6 μmol/g wet body mass	0.2 μmol/g wet body mass	7 days	Mehner and Wieser 1994
Roach (*R. rutilus*)	0.92 mg/g wet body mass	0.28 mg/g wet body mass	4 weeks	Mendez and Wieser, 1993
Perch (*P. flavescens*)	69 μmol/μg DNA	43 μmol/μg DNA	7 weeks	Foster and Moon, 1991
Atlantic salmon (*S. salar*)	23 mmol/l	13 mmol/l	Over winter	Brobbel et al., 1996

more depressed at warm compared to cold temperatures in juvenile rainbow trout (Kieffer and Tufts, 1998). It has also been demonstrated that the decreases in glycogen following fasting are size-dependent (Kieffer and Tufts, 1998), whereby, glycogen levels of smaller fish are generally more labile than they are in larger fish (Black et al., 1966; Scarabello et al., 1991; Kieffer and Tufts, 1998).

Decreased glycogen levels can ultimately set limits to burst performance in fish. Fasted fish display a lower anaerobic capacity (i.e. production of lactate) compared to fed fish (Scarabello et al., 1991). Interestingly, however, the post-exercise recovery rates for lactate and glycogen were not significantly different between starved and fed trout (Scarabello et al., 1991). Recently, however, it has been shown that fed striped bass returned their glycogen levels to control values post-stress (e.g. a 5 min netting stress) more rapidly than bass fasted for 3 days (Reubush and Heath, 1996). Differences in recovery rates may be related to the possible effects fasting has on the non-bicarbonate buffering capacity; however, little is known about this (see Nelson and Magnuson, 1987; Brobbel et al., 1996).

Although many studies have shown the potential impact of food deprivation on the muscle glycogen levels of fish, it is also noteworthy that fasting does not influence all the white muscle energy metabolites examined. In particular, the levels of white muscle PCr and ATP remain conserved during food deprivation (Scarabello et al., 1991; Kieffer and Tufts, 1998). This result is meaningful since it is well known that these two metabolites are utilized prior to glycogen as fuels for burst activity, and are important in maintaining burst activity (<15 s; Dobson and Hochachka, 1987). Therefore, the metabolites required for short-term burst activity (i.e. during predator–prey interactions) are not compromised because of fasting. Thus, it is highly possible that high energy phosphates may be 'spared' during food deprivation to maintain the fish's prey capturing or predator evasion capacity. Thus, limits to burst activity are probably set by the effects of fasting on the white muscle glycogen levels, rather than on the levels of adenylates and phosphagens.

Beyond their biological implications, these findings (i.e. Scarabello et al., 1991; Kieffer and Tufts, 1998) may be extremely valuable in the interpretation of exhaustive exercise data. In particular, the above discussion clearly shows that there is a strong influence of basal glycogen levels on the post-exercise metabolic status and the limits to burst activity in fish. Thus, in the future, it may be important to consider the physiological effects of short-term food deprivation when interpreting results from various studies or in designing protocols for experiments. This may be particularly the case when using small fish, since they may be more affected by short-term fasting compared with large fish.

From the above analysis, it is clear that most of the work to date has focused on the influence of food quantity and not the quality of food (i.e. carbohydrate versus protein diets). Recent literature (i.e. McKenzie et al., 1996), however, suggests that this may not be important. For example, Tilapia fed a diet enriched with different lipid compositions (i.e. saturated versus unsaturated) did not differ in the post-exercise oxygen consumption rates or muscle lactate concentrations (McKenzie et al., 1996). However, whether the same response would occur if fish were fed high protein or carbohydrate diets is not well understood. This would be an interesting area for future research.

4.4. Training

Much work has been carried out on exercise in fish (see Beamish, 1978; Wood, 1991; Milligan, 1996). Given that fish can be forced to swim at known speeds, they make ideal subjects for training studies (see Davison, 1997). Although some changes in the physiology, biochemistry and morphology have been noted in trained fish (see Davison, 1997), these results are often difficult to extrapolate to fish exercise performance. First, it is known that there is a great deal of intraspecific and interspecific variation in the response to training (Davison, 1997). Second, for a variety of reasons, most training studies have focused on endurance exercise training (Davison, 1997) and only recently (Pearson et al., 1990) has there been any effort to describe the effects of sprint training on aspects of fish biology (Gamperl et al., 1988; Pearson et al., 1990). It is reasonable to assume that these two training regimes will modify the physiology and biochemistry of fish differently and, therefore, may reflect differences in swimming performance. Therefore, to highlight any meaningful influence that training may have on

Table 5
Effect of training on various physiological parameters in fish[a]

Parameter	Effect	Reference
Heart size	T > U	Hochachka, 1961[b]
	T = U	Farrell et al., 1990[c]
	T = U	Houlihan and Laurent, 1987[d]
Increased blood hemoglobin concentration	T > U	Hochachka, 1961[b]
	T = U	Davie et al., 1986[e]
Number of capillaries per white muscle fiber	T > U	Davie et al., 1986[e]
Resting oxygen consumption rate rates	T < U	Woodward and Smith, 1985[f]
Post-exercise oxygen consumption rates	T > U	Hochachka, 1961[b]
Reliance on fatty acids	T > U	Davison, 1997[g]
Protein turnover rates	T > U	Houlihan and Laurent, 1987[d]
White muscle fats	T > U	Totland et al., 1987[h]
Resting plasma glucose levels	T > U	Johnston and Moon, 1980[i]
	T > U	Woodward and Smith, 1985[f]
	T = U	Hammond and Hickman, 1966[j]
Resting white muscle glycogen levels	T > U	Davison and Goldspink, 1977[k]
	T > U	Totland et al., 1987[h]
	T > U	Pearson et al., 1990[l]
	T = U	Johnston and Moon, 1980[i]
	T = U	Wendt and Saunders, 1973[m]
Resting white muscle PCr levels	T = U	Lackner et al., 1988[n]
	T = U	Pearson et al., 1990[l]
Resting white muscle ATP levels	T = U	Pearson et al., 1990[l]
Plasma cortisol levels	T < U	Woodward and Smith, 1985[f]
	T < U	Young and Cech, 1994[o]
Plasma catecholamine levels	T < U	Woodward and Smith, 1985[f]
Post-exercise muscle lactate levels	T > U	Hammond and Hickman, 1966[j]
	T > U	Lackner et al., 1988[n]
	T > U	Pearson et al., 1990[l]
Post-exercise muscle lactate recovery rate	T > U	Lackner et al., 1988[n]
	T > U	Pearson et al., 1990[l]
	T > U	Hammond and Hickman, 1966[j]
Post-exercise muscle glycogen recovery rate	T > U	Hochachka, 1961[b]
	T > U	Pearson et al., 1990[a,l]
Post-exercise muscle ATP recovery rate	T > U	Pearson et al., 1990[l]

[a] T, trained fish; U, untrained fish. Represents a pattern, however, not statistically significant.
[b] Fish trained at 30cm/s for 6 months; continuous swimming.
[c] Fish trained at 30 cm/s for 28–50 days.
[d] Fish trained at 1 body length/s for 6 weeks; continuous swimming.
[e] Fish trained at 1 body length/s for zero, 3, 30 or 200 days; continuous swimming.
[f] Fish trained at 1.5 body length/s for 6 weeks; intermittent swimming-fish trained for 8 h/day, 5 days /week for 6 weeks.
[g] Review article; data not provided.
[h] Fish trained at various speeds; ranging from about 0.28 to 0.45 body lengths/s for 8 months.
[i] Fish trained at 3 body lengths/s for 3 weeks; continuous swimming except 15 min per day for feeding purposes.
[j] Fish trained at 20 or 40 cm/s for 16 days; continuous swimming.
[k] Fish trained at 1.5, 3.0 or 4.5 body lengths/s for 28 days, 28 days or 14 days, respectively; continuous swimming.
[l] Fish were sprint trained by individual chasing for 30 s on alternate days for 9 weeks.
[m] No information on training regime.
[n] Fish trained at 3–5 body lengths/s for more than 2 months; intermittent training.
[o] Fish trained at either slow (0.5–1.2 body lengths/s) moderate (1.5–2.4 body lengths/s) or fast (2.4–3.6 body lengths/s) for 60 days; continuous swimming except for about 40 min. each day for fish feeding and tank cleaning.

exhaustive exercise capacities in fish, I have combined the effects of sprint and endurance training under a single heading (see Table 5).

Many studies have shown that endurance exercise training modifies myotomal muscle structure, enzyme activities and aerobic capacities in fish (Johnston and Moon, 1980; Lackner et al., 1988; Davison, 1997). These structural and physiological changes, and the noted changes in energy reserves (Table 5), suggest that trained fish may have greater stamina and may be better able to perform and recover from burst exercise

(Hochachka, 1961; Hammond and Hickman, 1966; Nahhas et al., 1982; Lackner et al., 1988; Pearson et al., 1990).

Overall, the physiological response to exhaustive exercise is greater in trained compared to untrained fish (see Table 5). For example, conditioned rainbow trout used more of their glycogen reserves compared to unconditioned fish (Hochachka, 1961). Although not statistically significant, a similar trend exists for juvenile rainbow trout (Pearson et al., 1990). Conditioned fish also sustained a higher post-exercise oxygen debt (three times that of unconditioned fish; Hochachka, 1961). Other studies suggest that, immediately following exhaustive exercise, the lactate load is much higher in trained versus untrained fish (Hammond and Hickman, 1966; Lackner et al., 1988; Pearson et al., 1990). Trained rainbow trout performed better (i.e. swam longer or swam further, respectively) compared with untrained fish (Hammond and Hickman, 1966; Nahhas et al., 1982; Pearson et al., 1990). Lastly, trained fish depleted less PCr (Lackner et al., 1988) and depleted less ATP (Pearson et al., 1990) following exercise compared to untrained fish.

Given that trained fish experience a greater physiological disturbance following exercise, one would expect that the post-exercise recovery period should be longer in trained fish. The available data indicates that trained fish recover more rapidly following exercise than untrained fish. For example, trained chub (*Leuciscus cephalus*) cleared post-exercise lactate loads about four times faster than untrained fish (Lackner et al., 1988). A similar trend was noted for trained juvenile rainbow trout (Pearson et al., 1990). Glycogen recovery rates were also much faster in trained versus untrained trout (Hochachka, 1961; Poston et al., 1969). In addition to clearance rates, it has been shown that trained fish increased exogenous glucose (Johnston and Moon, 1980; Woodward and Smith, 1985; Pearson et al., 1990) and have a greater reliance on lipids (Davison, 1997), both of which have been suggested as necessary to conserve endogenous energy reserves (i.e. glycogen) during the exercise bout or during the post-exercise recovery period. This information, although limited, may indicate that trained fish may have a greater array of metabolites to draw from during the recovery process.

Although the physiological response to exhaustive exercise is modified by training, the underlying basis for this phenomenon is still nebulous. It has been suggested that the accumulation of lactic acid and an inadequate buffering capacity may limit performance during exercise (Hochachka, 1961; Fig. 2). Thus, any advantage of training is presumably associated with an increased buffering capacity of the blood or muscle (see Pearson et al., 1990; Young and Cech, 1994), or may be related to enhanced enzyme concentrations (Davison, 1997) and/or an increase in the aerobic capacity of the swimming musculature (Lackner et al., 1988) of trained fish. However, the details of this are unclear to date and future research should focus on this area.

Whatever the mechanism(s) to explain the increased recovery rate in trained fish, these findings are interesting. Since training increases both the anaerobic capacity and the speed of recovery, training may set 'new physiological limits' for the fish. In particular, trained fish may be more metabolically prepared for additional bouts of exercise. This strategy would be particularly adaptive for migrating fish which have to negotiate different obstacles (e.g. fish ladders, waterfalls) during their upstream migration. Recent data indicate that recovery following angling was more rapid in migrating Atlantic salmon compared to other salmonid species (Booth et al., 1995). These results probably reflect the differences between wild and hatchery fish (i.e. trained versus untrained fish; Booth et al., 1995). However, adequate testing of this hypothesis would entail examining the physiological differences between wild and hatchery fish.

5. Discussion and areas for future research

Over the past forty years, there has been a large body of literature gathered on the physiological response to exhaustive exercise in fish. Most of this research has described this response and has focused on the mechanisms involved. Over the past 15 years, researchers have focused on the interspecific variability in this response, and the magnitude of the response between fish possessing different habitat and/or food requirements. More recently, however, it has been recognized that substantial intraspecific (i.e. within species) variability existed in the physiological response to

exercise, and this variability was almost as great as the variability noted between species. Until recently, however, the factors that governed this intraspecific variability were not clear and researchers have partially treated this variation as the result of inter-laboratory differences (e.g. husbandry differences, fish supply, exercise and sampling methodology).

This review has identified the role of several factors limiting the physiological response to exhaustive exercise in fish. Of the factors mentioned in this review, it is difficult to determine which factor contributes the greatest to the intraspecific variation in the response. Each factor influences various parts of the exercise process differently. For example, temperature plays a large role on the recovery process, whereas body size and nutritional status influence the stores of anaerobic fuels. In addition, it is not entirely clear how training influences the post-exercise response in fish, probably because of the considerable variability in the training regimes between studies. Furthermore, it is also possible that all four factors (and others not discussed in this review, such as potential genetic contributions; see Plaut and Gordon, 1994) interact with each other. Therefore, teasing out the exact role of each factor would be logistically challenging.

Despite these potential shortcomings, it is clear that many factors potentially contribute to the source of intraspecific variability noted in the literature. An important question that needs to be addressed is 'what gaps still exist in the literature?' Most work, to date, has focused on the importance of carbohydrate, adenylate and phosphogen metabolism in exhausting swimming, and how these energy reserves change during the recovery period. To gather tissues at the appropriate sampling times, researchers typically confine their animals to blackened perspex boxes. This technique has been used to acquire reasonable control values (see Wang et al., 1994a) and to standardize the techniques between laboratories. Although a common technique, confining a fish to a box may limit the animal's movement, which may have profound effects on the metabolite levels before and after the exercise protocol. The post-exercise recovery time, for example, may be significantly reduced if the fish has a chance to swim following burst exercise. Recently, Milligan et al., (2000) showed that muscle glycogen was completely resynthesized and muscle lactate cleared within 2 h of exercise in fish that were allowed to swim at low velocities following exhaustive exercise. This compared with a recovery time of greater than 6 h in fish that were held in still water. Thus, the impact of swimming on metabolic recovery is an important area for future research.

A second area for future research could be to broaden our knowledge of the fuels required for metabolic recovery. Traditionally, researchers have focused on the fate of lactate and the mechanisms behind lactate removal and glycogen repletion (see Moyes and West, 1995; Milligan, 1996). Recent evidence suggests that amino acids and fatty acids are used to support the post-exercise recovery process (Milligan and Girard, 1993; Wang et al., 1994b; Milligan, 1997). It may be possible, that under certain conditions lipids may be the preferred substrate to support the recovery process in fish, thus potentially impacting the recovery rates of the other metabolites. Although some work has been done on this, researchers should determine the contribution of lipids and proteins on the post-exercise recovery process in fish.

Recently, there has been some debate among fish physiologists as to when a fish has recovered from exercise. For most of us, a statistical test indicates recovery, even though the post-exercise values appear to be quite different from the control (resting) animals. In addition, recovery is also dependent on which metabolite is measured (see Moyes and West, 1995). Most emphasis has been placed on lactate recovery, which normally takes between 6–12 h to return to control values (see Section 1). How important is it for the fish to remove all of its lactate? Should the fish try to re-pay the 'post-exercise oxygen debt' quickly and/or replenish it's PCr and ATP stores? These questions test different levels of organization (e.g. whole body versus cellular approach), and therefore, provide fundamentally different answers. Answers to these questions, however, would provide a more comprehensive framework in which to examine both the ecological and evolutionary significance of burst activity in the life-history of fish.

Lastly, there is some controversy about the genetic contribution to the intraspecific variation noted in the physiological response to exercise in fish. Although fish physiologists have raised the issue, little research has been carried out in this area. Of the available data, Plaut and Gordon

(1994) report that the variation in the post-exercise lactate load in cloned zebra fish was approximately the same as that in wild fish. The results of this single study may suggest that the genetic component to this intraspecific variability may be small. However, more research is required to fully understand the potential role of genetics in the physiological response to exercise in fish.

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