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Two Fast-Type Fibers in Claw Closer and Abdominal Deep Muscles of the Australian Freshwater Crustacean, *Cherax destructor*, Differ in Ca^{2+} Sensitivity and Troponin-I Isoforms

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ABSTRACT One type of fast fiber and two types of slow (slow-twitch, S_1 and slow-tonic, S_2) fibers are found in decapod crustacean skeletal muscles that differ in contractile properties and myofibrillar protein isoform compositions. In this study the structural characteristics, protein isoform compositions, and Ca^{2+}-activation properties of fast fibers in the claw closer (F_1) and abdominal deep flexor (F_2) muscles of *Cherax destructor* were analyzed. For comparison, myofibrillar protein isoform compositions of slow (long-sarcomere) fibers from claw and abdomen were also determined; our results indicate that the slow fibers in the claw closer were the slow-twitch (S_1) type and those in the abdominal superficial flexor were primarily slow-tonic (S_2) type. F_1 fibers had shorter resting sarcomere lengths (2.93 μm in unstretched fibers and 3.06 μm in stretched fibers) and smaller fiber diameter (256 μm) than F_2 fibers (sarcomere lengths 3.48 μm in unstretched and 3.46 μm in stretched; 747 μm diameter). Moreover, F_1 fibers showed a narrower range in sarcomere lengths than F_2 fibers (2.81 to 3.28 μm vs. 2.47 to 4.05 μm in unstretched fibers). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting showed that the fast fibers from claw and abdomen differed in troponin-I composition; F_1 fibers expressed two isoforms of troponin-I (TnI_1 and TnI_2) in approximately equal amounts, whereas F_2 fibers expressed primarily TnI_3 and lower levels of TnI_1. F_1 fibers were more sensitive to Ca^{2+}, as shown by higher pCa values at threshold activation ($pCa_{10}=6.50\pm0.07$) and at 50% maximum force ($pCa_{50}=6.43\pm0.07$) than F_2 fibers ($pCa_{10}=6.12\pm0.04$ and $pCa_{50}=5.88\pm0.03$, respectively). F_1 fibers also had a greater degree of cooperativity in Ca^{2+} activation, as shown by a higher maximum slope of the force-pCa curve ($n_{Ca}=12.98\pm2.27$ vs. 4.34 ± 0.64). These data indicate that there is a greater fast fiber-type diversity in crustacean muscles than was previously supposed. Moreover, the differences in activation properties suggest that the TnI isoform composition influences the Ca^{2+} sensitivity of the contractile mechanism. *J. Exp. Zool. 301A:588–598, 2004.*

INTRODUCTION

Skeletal muscles are composed of many different myofibrillar proteins and all, with the exception of actin, occur as multiple isoforms (Pette and Staron, 2001; Silverman et al., '87). Individual muscle fibers express certain assemblages of protein isoforms, resulting in functionally and biochemically different fiber types (Mykles, '97; Pette and Staron, 2001). Crustacean skeletal muscles have at least three fiber types: one fast type with short sarcomeres and two slow types with long sarcomeres (S_1 or slow-twitch and S_2 or slow-tonic) (Mykles, '97). The S_1 fibers are used for slow movements, whereas S_2 fibers are specialized for sustained force production tasks, such as those involved in postural movements

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(Mykles, '88). The fast fibers are capable of much faster contractions but produce less force and are less oxidative than slow fibers (Costello and Govind, '83). The three fiber types differ in the assemblages of isoforms of myofibrillar proteins, including troponin-I (TnI), -T (TnT), and -C (TnC), tropomyosin (Tm), paramyosin (P), and myosin heavy and light chains (Wnuk, '89; Medler and Mykles, 2003; Mykles et al., '98; Sohn et al., 2000; Mykles et al., 2002). Analysis of myofibrillar proteins from a variety of decapod species indicates that certain isoforms serve as useful markers for distinguishing fiber types: paramyosin$_1$ (P_1) and P75 for fast fibers and TnT_1 for S_2 fibers; S_1 fibers lack these isoforms (Mykles, '97; Silverman et al., '87).

The claw closer muscle of the Australian freshwater crayfish, *Cherax destructor*, contains fast and slow fiber types that differ in sarcomere length and Ca^{2+}-activation properties. The fast fibers, located primarily in the central region of the closer muscle, have shorter sarcomeres and are less sensitive to Ca^{2+} than the slow fibers located peripherally (West, '97; West and Stephenson, '93; West et al., '92). For this study, the Ca^{2+} activation properties, morphologies, and myofibrillar protein isoform compositions of fast (short-sarcomere) fibers in the claw and abdominal muscles from *Cherax destructor* were analyzed. The myofibrillar protein isoform compositions of slow fibers from claw closer and abdominal superficial flexor muscles were also analyzed for comparison. Our results indicate that the short-sarcomere fibers in the claw and abdomen constitute two discrete types of fast fiber, termed F_1 and F_2, respectively.

MATERIALS AND METHODS

Experimental animals

Adult male and female yabbies, *Cherax destructor* (Clark), with an ocular carapace length of between 25 and 45 mm, were obtained from the Heathcote Yabby Farm (Victoria, Australia). Yabbies were housed in separate containers with gravel on the bottom, held at room temperature (18–24°C), and fed several times a week on chopped carrot and raw chicken. All animals used in this study were in the intermoult stage as determined by the morphology of uropod setae (Burton and Mitchell, '87).

Measurements of sarcomere length and fiber diameter

Claws were perfused with a relaxing buffer containing 2 mM ATP, 3 mM $MgCl_2$, 150 mM propionic acid, 10 mM EGTA, and 20 mM Hepes (pH adjusted to pH 7.1 with 4 M KOH). Similarly, the third and fourth segments from the abdomen were isolated and bathed in relaxing buffer. Individual fibers were mechanically skinned under paraffin oil with fine jeweller's forceps as described (West and Stephenson, '93) and mounted between two stainless steel pins with surgical thread (Deknatel 10). One of the pins was attached to a force transducer (SensorNor AME–802) and the other to a micromanipulator. The fibers were stretched until just taut. Diameter was measured with an eyepiece graticule while fibers were still under oil. Fibers were then transferred to a small spectrophotometric vial containing relaxing solution A (see below). The sarcomere lengths of stretched and unstretched fibers were determined from the primary diffraction maxima produced by the beam of a He-Ne laser (Spectra Physics 136–04) passed through the preparations as described (West and Stephenson, '93).

Fiber diameter was measured from micrographs of muscle cryosections stained for mitochondrial NADH-tetrazolium reductase histochemistry as described (Mykles, '88). Mitochondria are largely restricted to the subsarcolemmal cytoplasm of fast fibers, thus facilitating the identification of individual fibers (Mykles, '88). The central region of the claw closer, which contains mostly fast fibers (West et al., '92), and the abdominal deep flexor muscle in the third and fourth segments, were analyzed. Fiber diameters were determined using the ''measurement of the lesser fiber diameter.'' This is defined as the maximum diameter across the lesser dimension of the fiber, and is designed to overcome potential distortion with fibers sectioned obliquely (Dubowitz et al., '85).

SDS-PAGE and immunoblotting

Fibers were glycerinated in 20 mM Tris-HCl (pH 7.4), 0.1 M KCl, 1 mM EDTA, 0.1% (v/v) Triton X–100 and 50% (v/v) glycerol for three hours at 4°C under continuous stirring to remove soluble proteins, dissolved overnight in 50–100 μl of SDS sample buffer, and stored at −20°C (Medler and Mykles, 2003). A total of 24 fibers from the claw closer muscles and 17 fibers from the abdominal deep muscles from two animals were analyzed.

Proteins (4–6 μg) were separated by discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Bio-Rad (Hercules, CA) mini-Protean II slab gel apparatus (Medler and Mykles, 2003). Gels containing a 4% acrylamide stacking gel and a 12% separating gel were prepared from a 30% (w/v) N,N'-methylene bisacrylamide stock solution. Broad range molecular weight markers from Sigma (catalog #C3437) and BioRad (catalog #161–0317) were used. Immediately after electrophoresis, gels were either fixed in 10% glutaraldehyde and stained with silver or transferred to a polyvinylidine fluoride (PVDF) membrane (Medler and Mykles, 2003).

Immunodetection of TnI and TnT was carried out as described (Medler and Mykles, 2003; Sohn et al., 2000; Mykles et al., 2002). Briefly, PVDF membranes were incubated with TnI_3 antibody (1:5,000 dilution) or TnT antibody (0.8 μg IgG/ml) in TTBS (0.05 % Tween–20 in Tris-buffered saline; 20 mM Tris-HCl, pH 7.5, and 0.5 M NaCl) containing 3% non-fat milk. Although the antibodies were raised against lobster protein, they cross-react with the same proteins in other crustacean species (Sohn et al., 2000; Brüstle et al., 2001; Mykles et al., 2002). Membranes were washed in TTBS and incubated with biotinylated anti-rabbit IgG (1:10,000 dilution in TTBS) for one hour. This was followed by incubation with avidin/biotinylated horseradish peroxidase complex (Vectastain ABC reagent, 1:10,000 dilution in TTBS; Vector Labs, Burlingame, CA) and detection using chemiluminescence (Medler and Mykles, 2003).

Buffer solutions for skinned fibers

Solutions were prepared according to the methods of Ashley and Moisescu ('77) and West and Stephenson ('93). Solution A was a relaxing solution containing, in mM: EGTA, 50; $[Mg^{2+}]_{total}$, 10.30; Na^+, 36; K^+, 117; $[ATP]_{total}$, 8; creatine phosphate, 10; Hepes, 60; and sodium azide, 1. Solution B was an activating solution containing, in mM: EGTA, 50; $[Ca^{2+}]_{total}$, 49.5; $[Mg^{2+}]_{total}$, 8.12; Na^+, 36; K^+, 117; $[ATP]_{total}$, 8; creatine phosphate, 10; Hepes, 60; and sodium azide, 1. The free EGTA concentration in solutions A and B was measured by pH-metric titration (Moisescu and Pusch, '75). The apparent binding constant (K_{app}) between EGTA and Ca^{2+} of $4.78 \times 10^6\ M^{-1}$ (Moisescu and Thieleczek, '78) was used to determine the free Ca^{2+} concentrations (Moisescu and Pusch, '75). Solution B contained an estimated free Ca^{2+} concentration of 20 μM (pCa= 4.7), which induced maximum Ca^{2+}-activation in all fibers. Solutions containing pCa values between 7.00 and 4.7 were obtained by mixing solutions A and B in the required proportions. Solution H was a ''pre-activating'' solution containing, in mM: HDTA (1–6–diaminohexane-N-N-N′-N′-tetraacetic acid) 49.8; $[Mg^{2+}]_{total}$, 8.51; Na^+, 36; K^+, 117; $[ATP]_{total}$, 8; creatine phosphate, 10; Hepes, 60; and sodium azide, 1, which facilitated the rapid activation of the skinned fiber when exposed to Ca^{2+} (Moisescu and Thieleczek, '78). In all solutions pH was 7.10 ± 0.01; the concentration of ionized Mg^{2+} was maintained at 1.0 mM and did not vary by more than 10% between solutions. All solutions contained 130 mM sucrose in order to equal the osmotic pressure of the haemolymph. All experiments were conducted at room temperature (23–25°C).

Ca^{2+}-activation properties

The most convenient method of determining the effect of Ca^{2+} on force activation is a graphical representation of the relative force (P/P_0), where P is the steady-state force level at a given $[Ca^{2+}]$ and P_0 is the maximum Ca^{2+}-activated force, as a function of pCa ($-\log_{10}[Ca^{2+}]$). This relationship between P/P_0 and $[Ca^{2+}]$ can be described by a symmetrical sigmoid curve given by the Hill equation (Hill, '10):

$$P/P_0 = K[Ca^{2+}]^{nCa}/(1+K[Ca^{2+}]^{nCa})$$

where n_{Ca} is the Hill coefficient and K is a constant which is related to the pCa value corresponding to 50% P_0 (pCa_{50}) by the expression:

$$\log_{10}K = npCa_{50}$$

All data in this study have been presented with relative force expressed as a function of pCa. Curves were fitted to the experimental data using a Marquardt non-linear regression algorithm. Several quantitative characteristics, described in detail elsewhere (West et al., '99), can be derived from these curves. Briefly, the pCa_{10} represents the pCa value that produces 10% of the maximum Ca^{2+}-activated force and therefore gives an indication of the ''threshold'' level of Ca^{2+} required to elicit a force response. The pCa_{50} is the pCa value producing 50% of the maximum force providing a measure of the sensitivity of the contractile apparatus to Ca^{2+}. The Hill coefficient (n) is proportional to the maximum slope of the sigmoidal curves relating P/P_0 to pCa.

Data presentation and statistical analysis

All data are presented as mean ± SEM (n=number of fibers from 1–3 individuals). Statistical differences between means were determined with an unpaired t-test ($p < 0.05$).

RESULTS

Muscle anatomy and fiber morphology

The closer muscle in the claw of *Cherax destructor* is composed of a large pinnate muscle with the fibers oriented parallel to each other and extending from the dactyl apodeme to the inner surface of the exoskeleton. It is composed of a mixture of long-sarcomere (slow) and short-sarcomere (fast) fibers in a 58:42 ratio, with the majority of fast fibers located in the central region (West, '97; West et al., '92). The abdominal deep flexor muscle constitutes the bulk of the abdominal muscle mass and is composed entirely of short-sarcomere fibers. The individual fibers in the abdominal deep muscles have a unique orientation distinctly different from the pinnate array observed in the claw. The fibers are anchored at discrete points that appear as thin opaque bands within the muscle bundle. Bundles of fibers oriented in one direction meet with bundles oriented in a different direction at these points. Thus, the fibers run in many different orientations within the deep flexor muscle. In contrast, the fibers in the superficial flexor have a regular orientation and generally run parallel to the long axis of the abdomen. They are composed entirely of long-sarcomere fibers.

Fast fibers in the abdomen had a significantly larger diameter (about three-fold) than in the claw. The mean diameter of fast fibers in the abdominal deep flexor (F_2) was 747 ± 35.2 μm (n=25 from one individual). Mean diameter of fast fibers from the claw closer muscle (F_1) was 256 ± 73.6 μm (n=406 from three individuals).

Sarcomere length measurements on unstretched fibers showed that F_2 fibers from the abdominal deep flexor muscle also had significantly longer sarcomeres (3.48 ± 0.01 μm, n=27 from 2 abdominal segments in 1 individual) than F_1 fibers from the claw (2.93 ± 0.00 μm, n=30 from 2 individuals). Sarcomere lengths displayed a greater range in the F_2 fibers (2.47 to 4.05 μm) than in the F_1 fibers (2.81 to 3.28 μm). Most of the F_1 fibers had sarcomere lengths between 2.81 and 3.0 μm, whereas most F_2 fibers had sarcomere lengths between 3.21 and 4.0 μm (Fig. 1). A similar finding was obtained with the sarcomere lengths of the fibers used in the skinned fiber experiments, in which a small amount of tension was placed on the fibers. F_2 fibers had significantly longer sarcomeres (3.46 ± 0.08 μm, n=10 from 2 individuals) than F_1 fibers (3.06 ± 0.07 μm, n=11 from 3 individuals).

Analysis of myofibrillar proteins

A representative SDS-polyacrylamide gel of myofibrillar proteins from individual fast and slow fibers from the claw and abdominal muscles of *Cherax destructor* is shown in Figure 2. The results are consistent with all the fibers (24 from claw and 17 from abdomen) that were analyzed. The fast fibers in both claw (Fig. 2A, lane d) and abdomen (Fig. 2A, lane a) expressed a 110–kDa paramyosin isoform (P_1), TnT_2, and the P75 regulatory protein (75 kDa) characteristic of fast fibers in other decapod species (Mykles, '97). Slow fibers expressed P_2 (105 kDa) and TnT_3 but not P75 (Fig. 2A, lanes b, c, e-i). Fast and slow fibers also differed in the isoform distributions of TnI and tropomyosin; the tropomyosin in fast fibers had a slightly higher electrophoretic mobility than tropomyosin in slow fibers (Fig. 2A, compare lanes a, d with lanes b, c, e-i). It is well documented that crustacean skeletal muscles contain two types of slow fibers (S_1 and S_2), which are distinguished by the presence or absence of TnT_1 (Mykles, '97). The fibers found in *Cherax* were no different. The S_2 fibers in the abdominal superficial flexor muscle expressed TnT_1 in varying amounts (Fig. 2, lanes b, c; asterisks), whereas the S_1 fibers in the claw muscle lacked TnT_1 (Fig. 2, lanes e-i). The TnI isoforms also differed between the S_1 and S_2 fibers, but they were not analyzed in detail (Fig. 2A, compare lanes b, c with lanes e-i). The fast fibers from the claw and abdomen had identical myofibrillar protein compositions, except for TnI isoforms. The F_1 fibers expressed two isoforms (TnI_1 and TnI_2) of approximately 31 kDa in equal amounts (Fig. 2A, arrowheads in lane d; Fig. 3, lanes a-f), while the F_2 fibers expressed primarily TnI_3 (29 kDa) and a small amount of TnI_1 (Fig. 2A, arrowheads in lane a; Fig. 3, lanes g, h).

Ca^{2+}-activation properties

Fast fibers from the claw and abdomen differed in their Ca^{2+}-activated properties. As increased sarcomere length increases Ca^{2+} sensitivity of activation (Gordon et al., 2000), fibers with similar sarcomere lengths were selected; the mean

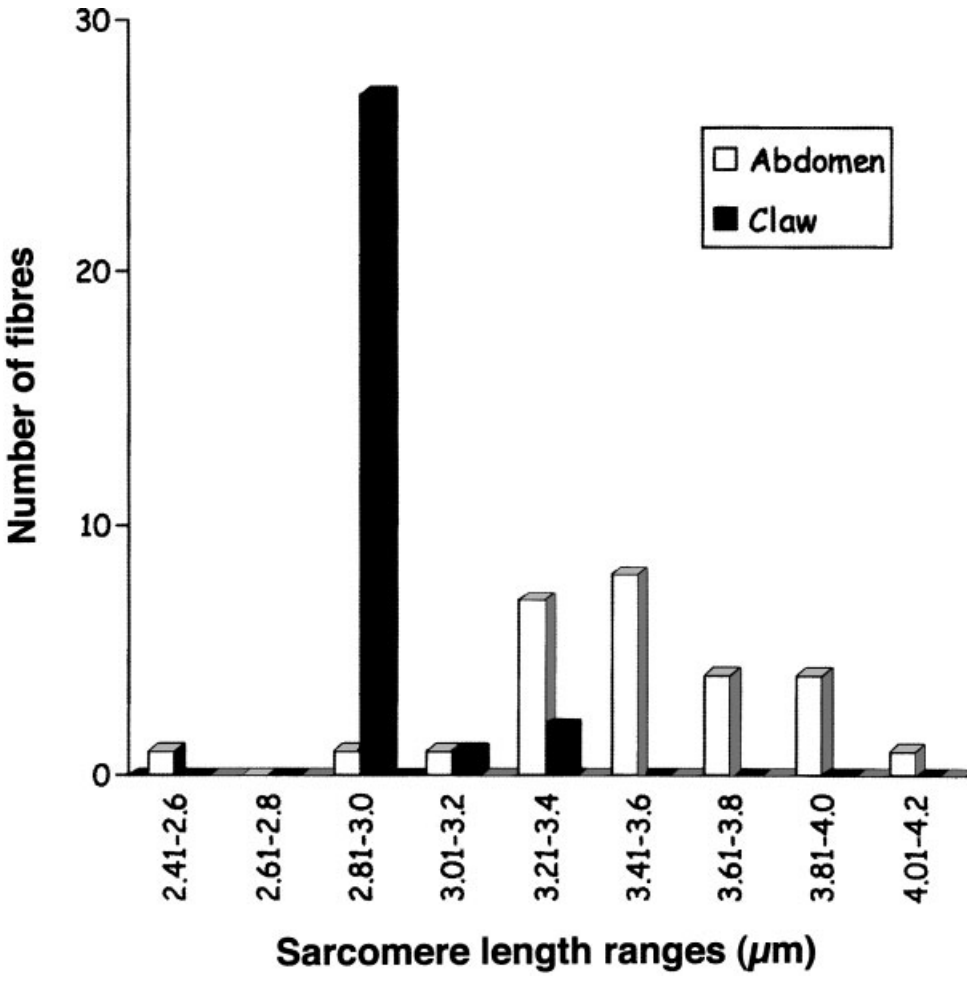

Fig. 1. Distribution of sarcomere lengths of the fast fibers from claw closer and abdominal deep flexor muscles of *Cherax destructor*. The range in sarcomere lengths was greater for the F_2 fibers in the abdomen than the F_1 fibers in the claw. Most of the F_1 fibers had sarcomere lengths between 2.81 and 3.0 μm and most of the F_2 fibers had sarcomere lengths between 3.21 and 4.0 μm.

sarcomere lengths of the F_1 fibers used for these measurements were on average 9–12% shorter than the F_2 fibers. Figure 4 shows representative force-pCa curves of the F_1 fibers from the claw closer and the F_2 fibers from the abdominal deep flexor muscle. The Ca^{2+}-activation properties of all fibers are summarized in Table 1. F_1 fibers were more sensitive to Ca^{2+}, as indicated by significantly higher pCa means at 10% (threshold activation), 50%, and 90% maximal force than the F_2 fibers. For example, the Ca^{2+} concentration needed to achieve 50% maximal force was about 3.5–fold higher for F_2 fibers (1.318 μM) than for F_1 fibers (0.372 μM). The maximum slope of the force-pCa curve (n_{Ca}) in F_1 fibers was significantly greater than that of the F_2 fibers, indicating a higher level of Ca^{2+}-binding co-operativity in the F_1 fibers. This difference in co-operativity leads to a greater difference (about 4.8–fold) in the Ca^{2+} concentration needed to achieve 90% maximal force (0.501 μM for F_1 vs. 2.399 μM for F_2) than that needed for 50% maximal force.

DISCUSSION

This is the first report of two biochemically-distinct types of fast fiber in a decapod crustacean, although previous studies have suggested that more than one fast fiber type may exist. Two types of fast fiber in lobster cutter claw were identified histochemically, based on different oxidative capacities (Costello and Govind, '83). A bundle of fast fibers in the antennal remoter muscle of lobster (*H. americanus*) contracts at high frequencies and has a sarcoplasmic reticulum that occupies about 60% of the fiber volume; these properties

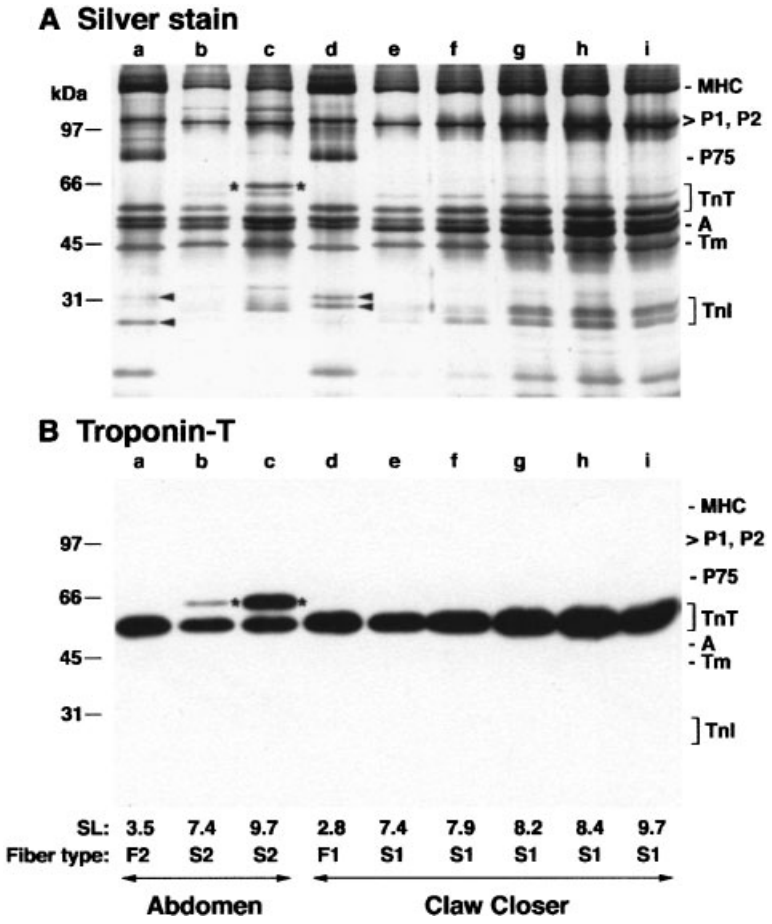

Fig. 2. SDS-PAGE (A) and troponin-T immunoblot (B) of myofibrillar proteins from fast and slow fibers in claw and abdominal muscles from *Cherax destructor*. The S_2 fibers in abdominal superficial muscle (lanes b and c) expressed TnT_1 (asterisks) and TnT_3, whereas the S_1 fibers in claw closer muscle (lanes e-i) expressed only TnT_3; both slow fiber types expressed P_2 (105 kDa). The fast fibers (F_1 in claw closer muscle and F_2 in abdominal deep muscle) expressed paramyosin$_1$, TnT_2, and P75 regulatory protein (75 kDa), but differed in expression of troponin-I isoforms (lanes a and d, arrowheads). Positions of molecular weight standards indicated at left. Abbreviations: A, actin; MHC, myosin heavy chain; P, paramyosin; P75, P75 regulatory protein; SL, sarcomere length (μm); Tm, tropomyosin; TnI, troponin-I; and TnT, troponin-T.

distinguish it from the fast fibers in the cutter claw closer and the abdominal deep muscles (Bevengut et al., '93). The maxilliped flagellum adductor muscle in the blue crab, *Callinectes sapidus*, also contracts at high frequency and is highly fatigue-resistant, which corresponds to a high concentration of mitochondria in the subsarcolemmal cytoplasm of the muscle fibers (Silverman and Charlton, '80). Three distinct types of ''phasic'' fibers (type II, III, and IV) in the leg closer muscle of the crab *Eriphia spinifrons* differ in pH lability of myofibrillar ATPase and force-pCa relationship (Galler and Rathmayer, '92). However, the three fiber types have long (at least 6 μm) sarcomeres (Read and Govind, '93) that are characteristic of slow fibers (Galler and Neil, '94). None of these studies examined myofibrillar protein isoform compositions.

There is a strict segregation in the distribution in the two fast fibers, as F_1 fibers are only found in the claws and F_2 fibers are only found in the abdomen. Furthermore, ''hybrid'' fibers containing intermediate TnI isoform compositions are never observed. This is in contrast to the long-sarcomered (slow) fibers, in which S_1 and S_2 fibers can occur in the same muscle, and fibers with intermediate isoform assemblages are common. For example, the antennal, leg opener, and abdominal superficial muscles in crayfish and lobster, and claw closer muscle in land and fiddler crabs contain mixtures of S_1 and S_2 fibers (Mykles, '88, '97; Gruhn and Rathmayer, 2002; Bevengut

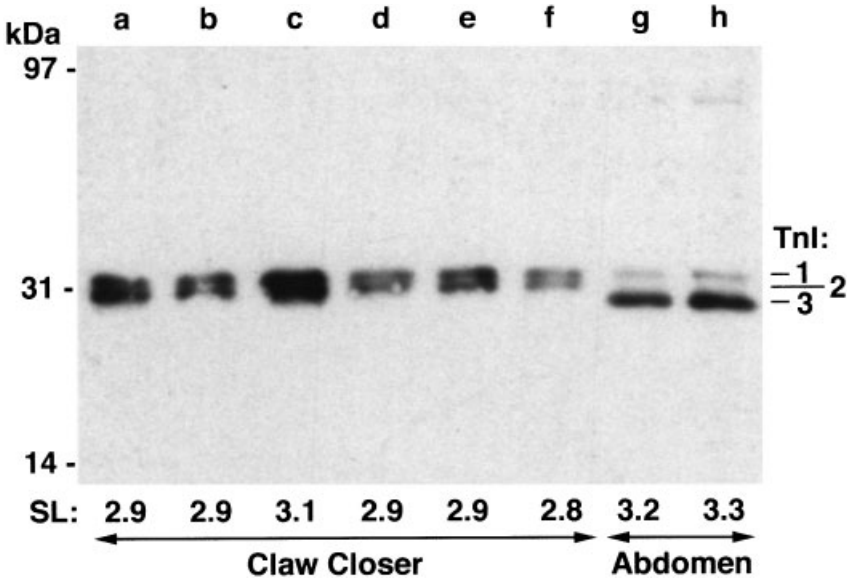

Fig. 3. Immunoblot analysis of troponin-I isoforms in fast fibers from claw closer and abdominal deep flexor muscles. F_1 fibers (lanes a-f) expressed TnI_1 and TnI_2, whereas F_2 fibers (lanes g and h) expressed primarily TnI_3 and lower levels of TnI_1. Positions of molecular weight standards indicated at left. Abbreviations: SL, sarcomere length (μm); TnI, troponin-I.

et al., '93; Mykles et al., 2002). Further analysis of single fibers in these muscles indicates that the slow phenotype forms a continuum, with the S_1 and S_2 fibers representing extremes of the range (Mykles, '88; Medler and Mykles, 2003; Sohn et al., 2000; Mykles et al., 2002;). Histochemical properties and myofibrillar protein isoform compositions of slow fibers are correlated with synaptic facilitation (Gruhn and Rathmayer, 2002; Mykles et al., 2002). Moreover, chronic electrical stimulation induces phenotypic changes in myofibrillar ATPase activity in crayfish abdominal superficial muscles (Gruhn and Rathmayer, 2002). These data indicate that slow fibers display phenotypic plasticity and that this plasticity is influenced by neuronal activity.

Like mammalian skeletal muscle, crustacean muscle fibers have an actin-linked Ca^{2+}-regulatory mechanism, in which specific assemblages of myosin, TnT, TnI, TnC, and tropomyosin determine Ca^{2+} sensitivity of force production (Perry, '98; Galler and Neil, '94; Galler et al., '97; Kögler et al., '98; Geiger et al., '99). Thus, the Ca^{2+}-activation profiles are a reflection of all the different proteins present in a fiber. Although several isoforms of myosin heavy chain, TnT, and tropomyosin have been identified in decapods, fast muscle fibers apparently express only one isoform of each (Medler and Mykles, 2003; Mykles et al., '98, 2002; Sohn et al., 2000). The electrophoretic mobilities of TnT and tropomyosin were identical in the two fast fiber types in *Cherax* (Fig. 2). These data suggest that the differences in calcium activation are not a consequence of different tropomyosin and TnT isoforms.

In mammalian skeletal muscle a critical step in the regulatory process involves a large increase in the affinity of TnC for TnI, triggered by the binding of Ca^{2+} to TnC (Grabarek et al., '92). Although the TnC isoform compositions of the different fiber types from *Cherax destructor* have not been analyzed, the Ca^{2+}-binding properties of two TnC isoforms (α and γ) from crayfish abdominal muscle have been determined (Kobayashi et al., '89). Both isoforms possess two Ca^{2+}-specific binding sites with different Ca^{2+}-binding properties but lack the high-affinity Ca^{2+}/Mg^{2+}-binding sites found in vertebrate TnC (Grabarek et al., '92). Binding of TnC α or γ with TnI or with a complex of TnI and TnT alters their affinity to Ca^{2+} in both binding sites (Wnuk, '89). Changes in Ca^{2+} sensitivity of skinned barnacle fibers after TnC extraction and reconstitution suggest that different TnI isoforms can influence Ca^{2+} regulation (Ashley et al., '91; Gordon et al., '97), and that interactions between the TnC, TnI, and TnT isoforms can alter the sensitivity of the regulatory system to Ca^{2+}. Barnacle troponin is thought to have very similar properties to crayfish troponin (Ashley et al., '91). Phosphorylation of TnI is associated with the potentiation of muscle contraction by proctolin in the isopod, *Idotea marginata*

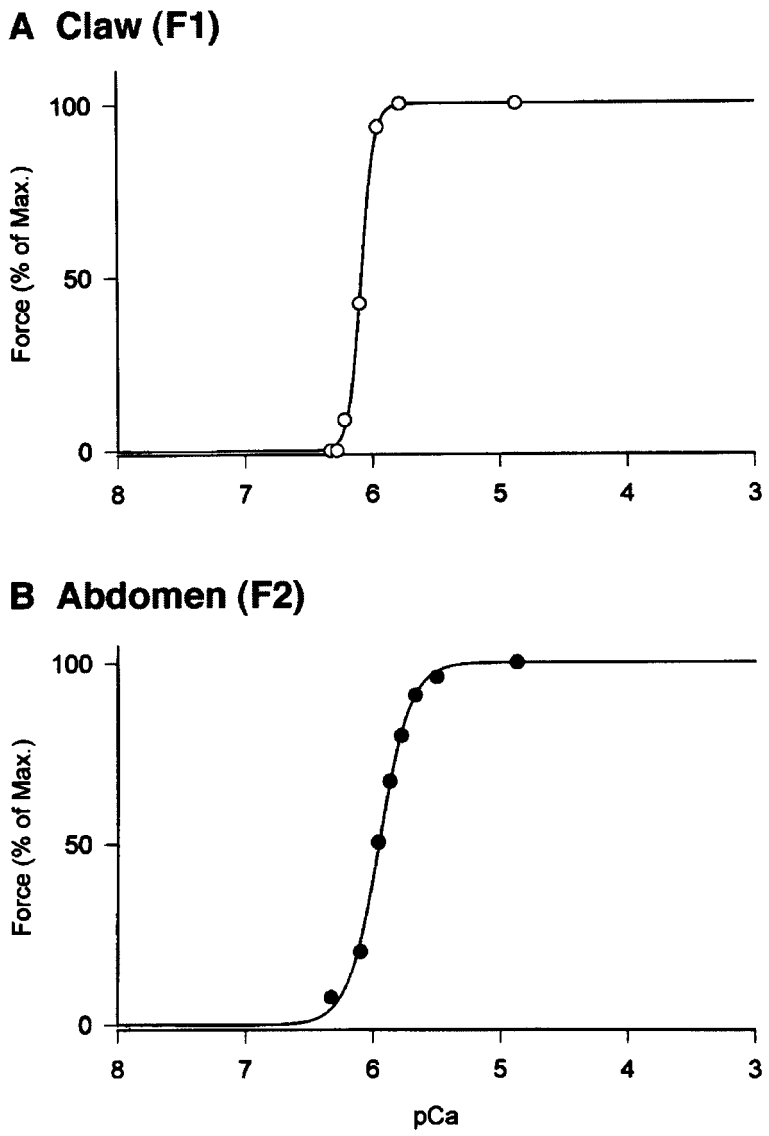

Fig. 4. Representative force-pCa curves comparing the Ca^{2+}-activation properties of fast fibers from the claw (A) and the abdominal deep flexor (B) muscles. The curve for the F_1 fiber was steeper and was shifted to a higher pCa than the curve for the F_2 fiber, indicating F_1 fibers were more sensitive to Ca^{+}. The curves were drawn through the data points by fitting the data with the Hill equation using least-squares regression (see Methods). Sarcomere lengths were 2.90 μm for (A) and 3.18 μm (B).

TABLE 1. Force-pCa parameters of F_1 (claw) and F_2 (abdominal deep flexor) fibers. Data presented as mean ± SEM; n=11 F_1 fibers from 3 animals or n=10 F_2 fibres from 2 animals. Fibers with similar sarcomere lengths were selected to minimize potential effects of sarcomere length on Ca^{2+} sensitivity. The sarcomere lengths of the skinned fibers were 3.06 ± 0.07 (11) for F_1 and 3.46 ± 0.08 (10) for F_2

Parameter	F_1 type	F_2 type
pCa_{10}	6.50 ± 0.07	6.12 ± 0.04*
pCa_{50}	6.43 ± 0.07	5.88 ± 0.03*
pCa_{90}	6.30 ± 0.05	5.62 ± 0.04*
n_{Ca}	12.98 ± 2.27	4.34 ± 0.64*

*Indicates the mean in F_2 fibers was significantly different from the mean in F_1 fibers ($p < 0.05$).

(Brüstle et al., 2001). These data indicate that, even if the two types of fast fiber in *Cherax* express the same TnC isoform, differences in the TnI isoforms can influence the properties of the troponin/tropomyosin complex sufficiently to explain the different Ca^{2+}-activation profiles shown in Figure 4 and Table 1.

The major differences in the activation profiles between the fast fibers found in the claw and abdomen were a greater sensitivity to Ca^{2+} and a significantly steeper force-pCa relationship in fast fibers from the claw (Fig. 4; Table 1). The F_1 fibers from the claw developed 50% of their maximum

tension at lower Ca^{2+} concentrations than F_2 fibers from the abdomen. This difference in sensitivity is not due to differences in sarcomere length, as fibers with similar sarcomere lengths were used. Moreover, the results are contrary to what would be predicted. As Ca^{2+} sensitivity is positively correlated with sarcomere length (Gordon et al., 2000), it is predictable that the F_2 fibers would have higher pCa values than the F_1 fibers, but this was not observed (Table 1). Sarcomere length is not necessarily an indicator of Ca^{2+} sensitivity in crustacean muscle fibers, as fibers with very different sarcomere lengths (short vs. long) can have similar Ca^{2+} activation properties (West and Stephenson, '93). The steeper force-pCa curves for the F_1 fibers indicate a greater degree of co-operativity compared to F_2 fibers. The shallower slope in the F_2 fibers allows defined changes in Ca^{2+}-activated force when the intracellular Ca^{2+} concentration is changed over a relatively wide range. These results suggest that larger changes in intracellular Ca^{2+} concentration are required to activate the F_2 fibers fully, and that F_2 fibers need a longer time for contraction and relaxation than F_1 fibers. Thus, the force responses may be slower in the F_2 fibers.

Sarcomere lengths in crustacean muscles cover a much broader range than those of vertebrate skeletal muscles. In crustaceans, fast muscles are composed of myofibrils with sarcomere lengths in the range of 2–4 μm, while those of slow muscles have sarcomere lengths from 6–12 μm (Silverman et al., '87). Although a general correlation exists between sarcomere length, speed of shortening, and myofibrillar isoform composition, a precise relationship among these variables has not been established (Silverman et al., '87). Nevertheless, there is good evidence that fibers with short sarcomeres will contract with greater speed than long-sarcomered fibers, if all other factors are equal. As discussed by Josephson ('75), the shortening velocities of sarcomeres in series are additive. That is, the higher the number of contractile units arranged in series, the faster the shortening velocity. In contrast, fibers with longer sarcomeres compromise shortening velocity for increased force development. More precisely, the amount of force developed should reflect the length of the myosin filaments (A-band), which is positively correlated with sarcomere length (Josephson, '75; Taylor, 2000). Confirming this prediction, measurements of forces developed by muscles with a range of sarcomere lengths demonstrates a 1:1 correlation between maximum muscle stress and resting sarcomere length (Taylor, 2000).

The significance of fast fibers with different Ca^{2+} activation properties and sarcomere lengths may relate to functional differences between the claw and abdominal deep muscles. The higher Ca^{2+} cooperativity and shorter and more uniform sarcomere length of F_1 fibers suggests that the F_1 fibers are designed both biochemically and structurally to contract with greater speed over a narrower Ca^{2+} concentration than the F_2 fibers. The lower Ca^{2+} cooperativity and broader range of sarcomere lengths in the F_2 fibers may reflect a greater range of contractile response in these muscles. The claw in *Cherax* is used in burrowing, prey capture, feeding, and aggressive behaviour, whereas the abdominal deep flexor is used in the tail flip escape response. The *Cherax* claw muscle contains both F_1 and S_1 fibers (West, '97; West and Stephenson, '93; West et al., '92), analogous to the fiber composition of lobster cutter claw (Medler and Mykles, 2003). The S_1 fibers contribute strength and endurance, whereas the F_1 fibers function in rapid closure of the dactyl (Atwood, '73). In contrast, the abdominal deep muscle is used less frequently; it produces a rapid series of powerful contractions that result in the animal moving rapidly backwards through the water (Baldwin et al., '99). In lobster (*H. americanus*), the muscles have essentially the same functions, but the fast fibers in the cutter claw and abdomen have identical isoform compositions (Medler and Mykles, 2003; Sohn et al., 2000). The Ca^{2+} sensitivities and sarcomere lengths may indicate a further degree of specialization of the fast fiber types in *Cherax* not found in lobster.

In summary, this study reports two distinct fast fiber types in the claw and abdominal muscles of *Cherax destructor*. As in other decapods, the fast fibers in *Cherax* express P_1 and the P75 protein and the two slow fibers were distinguished by the unique expression of troponin T_1 in the S_2 fibers. The only apparent difference in isoform composition between the F_1 and F_2 fibers was the TnI composition, which suggests that TnI isoforms influence the Ca^{2+} sensitivity of the fibers. However, it is possible that other myofibrillar proteins contribute to the differences in Ca^{2+} activation. Further work should determine whether isoforms of myosin heavy and light chains and TnC are differentially expressed in F_1 and F_2 fibers.

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LITERATURE CITED

Ashley CC, Moisescu DG. 1977. Effect of changing the composition of the bathing solutions upon the isometric tension-pCa relationship in bundles of crustacean muscles. J Physiol 270:627–652.

Ashley CC, Lea TJ, Hoar PE, Kerrick WGL, Strang PF, Potter JD. 1991. Functional characterization of the two isoforms of troponin C from the arthropod *Balanus nubilus*. J Muscle Res Cell Motil 12:532–542.

Atwood HL. 1973. An attempt to account for the diversity of crustacean muscles. Am Zool 13:357–378.

Baldwin J, Gupta A, Iglesias X. 1999. Scaling of anaerobic energy metabolism during tail flipping behaviour in the freshwater crayfish, *Cherax destructor*. Mar Freshwater Res 50:183–187.

Bevengut M, McTeague JA, Govind CK. 1993. Fiber composition of antennal muscles in the lobster *Homarus americanus* and the crayfish *Procambarus clarkii*. J Crustacean Biol 13:256–267.

Brüstle B, Kreissl S, Mykles DL, Rathmayer W. 2001. The neuropeptide proctolin induces phosphorylation of a 30 kDa protein associated with the thin filament in crustacean muscle. J Exp Biol 204:2627–2635.

Burton EM, Mitchell BD. 1987. Moult staging in the Australian freshwater crayfish *Cherax albidus* Clark and *Cherax destructor* Clark (Decapoda: Parastacidae), via uropod setal development. Aust J Mar Freshw Res 38: 345–352.

Costello WJ, Govind CK. 1983. Contractile responses of single fibers in lobster claw closer muscles: correlation with structure, histochemistry, and innervation. J Exp Zool 227:381–393.

Dubowitz V, Sewry CA, Fitzimons RB. 1985. Muscle Biopsy: A Modern Approach. 2nd Ed. Philadelphia: P.A. Saunders.

Galler S, Rathmayer W. 1992. Shortening velocity and force/pCa relationship in skinned crab muscle fibres of different types. Pflügers Arch 420:187–193.

Galler S, Neil DM. 1994. Calcium-activated and stretch-induced force responses in two biochemically defined muscle fibre types of the Norway lobster. J Muscle Res Cell Motil 15:390–399.

Galler S, Schmitt TL, Hilber K, Pette D. 1997. Stretch activation and isoforms of myosin heavy chain and troponin-T of rat skeletal muscle fibres. J Muscle Res Cell Motil 18:555–561.

Geiger PC, Cody MJ, Sieck GC. 1999. Force-calcium relationship depends on myosin heavy chain and troponin isoforms in rat diaphragm muscle fibers. J Appl Physiol 87: 1894–1900.

Gordon AM, Qian Y, Luo Z, Wang CK, Mondares RL, Martyn DA. 1997. Characterization of troponin-C interactions in skinned barnacle muscle: comparison with troponin-C from rabbit striated muscle. J Muscle Res Cell Motil 18:643–653.

Gordon AM, Homsher E, Regnier M. 2000. Regulation of contraction in striated muscle. Physiol Rev 80:853–924.

Grabarek Z, Tao T, Gergely J. 1992. Molecular mechanism of troponin-C function. J Muscle Res Cell Motil 13:383–393.

Gruhn M, Rathmayer W. 2002. Phenotype plasticity in postural muscles of the crayfish *Orconectes limosus* Raf.: Correlation of myofibrillar ATPase-based fiber typing with electrophysiological fiber properties and the effect of chronic nerve stimulation. J Exp Zool 293:127–140.

Hill AV. 1910. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J Physiol 40:iv-vii.

Josephson RK. 1975. Extensive and intensive factors determining the performance of striated muscle. J Exp Zool 194:135–154.

Kobayashi T, Takagi T, Konishi K, Wnuk W. 1989. Amino acid sequence of the two major isoforms of troponin C from crayfish. J Biol Chem 264:18247–18259.

Kögler H, Plathow C, Al-Hillawi E, Trayer IP, Caspar J. 1998. Replacement of troponin-I in slow-twitch skeletal muscle alters the effects of the calcium sensitizer EMD 53998. Pflügers Arch 436:398–406.

Medler S, Mykles DL. 2003. Analysis of myofibrillar proteins and transcripts in adult skeletal muscles of the American lobster *Homarus americanus*: variable expression of myosins, actin, and troponins in fast, slow-twitch, and slow-tonic fibres. J Exp Biol 206:3557–3567.

Moisescu DG, Pusch H. 1975. A pH-metric method for the determination of the relative concentration of calcium to EGTA. Pflügers Arch 355:R122.

Moisescu DG, Thieleczek R. 1978. Arthropod skeletal muscles — insensitive to Sr^{2+}. Proc Aust Physiol Pharmacol Soc 9:158P.

Mykles DL. 1988. Histochemical and biochemical characterization of two slow fiber types in decapod crustacean muscles. J Exp Zool 245:232–243.

Mykles DL. 1997. Crustacean muscle plasticity: Molecular mechanisms determining mass and contractile properties. Comp Biochem Physiol 117B:367–378.

Mykles DL, Cotton JLS, Taniguchi H, Sano KI, Maeda Y. 1998. Cloning of tropomyosins from lobster (*Homarus americanus*) striated muscles: fast and slow isoforms may be generated from the same transcript. J Muscle Res Cell Motil 19:105–115.

Mykles DL, Medler S, Koenders A, Cooper R. 2002. Myofibrillar protein isoform expression is correlated with synaptic efficacy in slow fibres of the claw and leg opener muscles of crayfish and lobster. J Exp Biol 205:513–522.

Perry SV. 1998. Troponin T: Genetics, properties and function. J Muscle Res Cell Motil 19:575–602.

Pette D, Staron RS. 2001. Transitions of muscle fiber phenotypic profiles. Histochem Cell Biol 115:359–372.

Read AT, Govind CK. 1993. Fine structure of identified muscle fibers and their neuromuscular synapses in the limb closer muscle of the crab *Eriphia spinifrons*. Cell Tissue Res 271:23–31.

Silverman H, Charlton MP. 1980. A fast-oxidative crustacean muscle: histochemical comparison with other crustacean muscle. J Exp Zool 211:267–273.

Silverman H, Costello WJ, Mykles DL. 1987. Morphological fiber type correlates of physiological and biochemical properties in crustacean muscle. Am Zool 27:1011–1019.

Sohn J, Mykles DL, Cooper RL. 2000. Characterization of muscles associated with the articular membrane in the dorsal surface of the crayfish abdomen. J Exp Zool 287: 353–377.

Taylor GM. 2000. Maximum force production: why are crabs so strong? Proc R Soc Lond Biol 267:1475–1480.

West JM. 1997. Ultrastructural and contractile activation properties of crustacean muscle fibres over the moult cycle. Comp Biochem Physiol 117B:333–345.

West JM, Barclay CJ, Luff AR, Walker DW. 1999. Developmental changes in the activation properties and ultrastructure of fast- and slow-twitch muscles from fetal sheep. J Muscle Res Cell Motil 20:249–264.

West JM, Humphris DC, Stephenson DG. 1992. Differences in maximal activation properties of skinned short- and long-sarcomere muscle fibres from the claw of the freshwater crustacean *Cherax destructor*. J Muscle Res Cell Motil 13:668–684.

West JM, Stephenson DG. 1993. Ca^{2+}- and Sr^{2+}-activation properties of skinned muscle fibers with different regulatory systems from Crustacea and rat. J Physiol 462:579–596.

Wnuk W. 1989. Resolution and calcium-binding properties of the two major isoforms of troponin C from crayfish. J Biol Chem 264:18240–18246.