

## Characterization of the muscle fibres types in a pristine and regenerate chela from the Christmas Island Red Crab *Geocarcoidea natalis*

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This study investigated the relationship between chelae morphology and muscle fibre composition of the Christmas Island Red crab. Males of this species grow larger and develop asymmetrical chelae; females however possess smaller symmetrical chelae. This species is purely terrestrial and use their chelae for various functions including, burrowing, defense, courtship, grooming and walking.

Crustacean muscle fibres show great variation in fibre type which can be categorized by their greatly different sarcomere length (SL). Short-sarcomere fibres have a  $SL \leq 4\mu\text{m}$ , a fast contraction speed, but produce relatively low forces. Fibres with long-sarcomeres have a  $SL > 6\mu\text{m}$ , exhibit a slower speed of contraction and develop considerably more force than short-sarcomere fibres (Atwood, 1976; West & Stephenson, 1993). Fibre types in crustaceans can also be distinguished by the contractile and regulatory protein isoforms they contain (Mykles, 1988; Koenders *et al.*, 2004). Short-sarcomeres can be distinguished from long-sarcomere fibres by the presence of P75 (only expressed in fibres with short sarcomeres); the isoforms of paramyosin (short-sarcomere P1; long-sarcomere P2) and in the troponin I and T isoforms (Koenders *et al.*, 2004).

Muscle fibre composition was determined in two morphologically distinct chelae types from male Red crabs. The large chela (propus and dactyl make contact only at the tips when chela is closed) and a regenerating chela (propus and dactyl have several points of contact when chela is closed). Chelipeds were removed at the basi-ischial joint, minimizing blood loss. Chelae were removed and thoroughly perfused with either 10% neutral buffered formalin or 70% ethanol. Fixed muscle fibres were dissected from specific regions in the lateral-interior surface of the pristine and regenerate chelae. SL of fibres was determined using a He-Ne laser and confirmed using histology. Protein isoforms of several fibres with greatly different sarcomere length were separated using SDS-PAGE.

72% of male crabs had the large chelae on the left hand side. A total of 766 fibres were dissected from 11 different areas of the pristine chela and 870 fibres from 9 areas in the regenerating chela and their SL determined. Fibres in both chelae exhibited a broad range of SL's ranging from 3-21 $\mu\text{m}$  (pristine) and 3-15  $\mu\text{m}$  (regenerate). The average SL of fibres was 9.66 $\mu\text{m}$  and 8.45 $\mu\text{m}$  in the pristine and regenerate chelae respectively. The number of fibres with a  $SL < 4\mu\text{m}$  was one in the pristine and twelve in the regenerate indicating that only a very small number of fast contracting fibres are present. Regenerate chela in other crustacean species, have a greater proportion of fibres with short-sarcomeres in the central portions of the chela. No such trend was observed in the Red crab. Fibres from the chelae with a sarcomere length just under 4 $\mu\text{m}$  do not express the proteins characteristically used to identify short-sarcomere fibres, namely P75. Thus the fibres in the chelae of the Christmas Island Red crab suggest a continuum in fibre type with the majority of fibres expressing proteins of the long-sarcomere fibre type but having a broad range of sarcomere lengths.

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