Sexual dimorphism of extensor carpi radialis muscle size, isometric force, relaxation rate and stamina during the breeding season of the frog *Rana temporaria* Linnaeus 1758

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Summary

Mating success of individual male frogs within explosive breeding species can depend on their ability to compete for a mate and to hold onto that mate during amplexus. Such importance of amplexus has resulted in the evolution of sexual dimorphism in the morphology and contractile characteristics of the anuran forelimb muscles used during amplexus. The aims of our study were to use an explosive breeding frog (*Rana temporaria*) during the breeding season to compare extensor carpi radialis (ECR) muscle length, mass, isometric activation times, relaxation times, absolute force, relative force (stress) and fatigue between male and female frogs. We found that ECR muscle mass and length were greater (tenfold and 1.4-fold, respectively), absolute tetanic muscle force and relative tetanic force (stress) were greater (16-fold and 2.2-fold, respectively) and relaxation times were slower in males than in females. Male ECR muscles incompletely relaxed during fatigue tests and showed less fatigue than female muscles. These sex differences are likely to be beneficial to the male frogs in allowing them to produce relatively high absolute muscle forces for prolonged periods of time to hold onto their mate during amplexus.

Key words: amplexus, contractile, fatigue, sex, morphology, tetanus.

Introduction

In male frogs reproductive success depends upon a combination of an individual's abilities to migrate to the breeding site, defend a territory, actively search for a mate, attract a mate *via* calling and compete with rival males for that mate (Arak, 1983; Duellman and Trueb, 1994; Irwin et al., 2003; Lee and Corrales, 2002; Wells, 1977). The relative importance of each of these behaviours depends upon the species, breeding male density, male size and sex ratios (Arak, 1983; Wells, 1977). In some species of explosive breeders such as *Rana temporaria* many more males than females are present in the breeding pond at any one time, resulting in competition between males. Many males may attempt to clasp (hold) the same female to try to ensure they copulate with her, or they will try to dislodge a male that is already grasping a female (Arak, 1983; Elmberg, 1991; Wells, 1977). In some species such competition can lead to intrasexual, interspecific or multiple amplexus (Marco and Lizana, 2002), and can even result in multiple paternity (Lode and Lesbarreres, 2004; Roberts et al., 1999). In higher male densities of explosive breeders, males of large body length appear to have an advantage that increases the likelihood of them mating (Arak, 1983; Elmberg, 1991; Lee, 2001); however, it now appears that

the real key to male mating success in such species may be greater absolute mass of adductor and flexor forelimb muscles, regardless of body size of the male (Lee, 2001).

Males of many frog species undergo seasonal changes in both size and contractile properties of the muscles used in amplexus to improve their performance. Flexor carpi radialis muscle from male *Rana temporaria* undergoes seasonal changes in contractile characteristics (Melichna et al., 1972) such that during the breeding season half twitch relaxation time and maximal rate of force development are both slower than during the summer. These alterations in contractile characteristics are linked to seasonal increases in gonadotrophin concentrations (e.g. LH, luteinizing hormone and FSH, follicle-stimulating hormone), which affect both amplexing behavior and muscle morphology (Itoh and Ishii, 1990). In *Rana esculenta*, amplexing males exhibit higher levels of testosterone and lower levels of estradiol than non-amplexing individuals (Gobbetti and Zerani, 1999), and in *Rana pipiens* testosterone increases forelimb muscle dry mass by more than 150% under experimental conditions (Kim et al., 1998). Testosterone treatment of castrated male *Xenopus laevis* frogs leads to increases in muscle size, force and twitch relaxation times in flexor carpi radialis, yet this treatment has little effect on the

hindlimb locomotor muscle ileofibularis (Regnier and Herrera, 1993). Testosterone treatment also increases the number of tonic fibres in coracoradialis muscle (another forelimb muscle used to clasp females) in *Xenopus laevis*, which could explain the seasonal prolongation in 'clasp' muscles of time to fatigue and relaxation time (Rubinstein et al., 1983).

The prevalence of amplexus as an important stage of the reproductive mode of anurans has resulted in the evolution of sexual dimorphism in the forelimb muscles of anurans (Clark and Peters, 2006; Oka et al., 1984; Peters and Aulner, 2000; Sidor and Blackburn, 1998; Yekta and Blackburn, 1992). Some forelimb muscles such as extensor carpi radialis, flexor carpi radialis, sternoradialis and abductor indicus longus that are involved in amplexus have been found to be larger in mass and cross-sectional area in male than in female frogs (Clark and Peters, 2006; Kirby, 1983; Oka et al., 1984; Peters and Aulner, 2000; Yekta and Blackburn, 1992) and to have more oxidative fibers and/or higher oxidative capacity in males than in females (Kirby, 1983; Melichna et al., 1972; Peters, 2001; Rubinstein et al., 1983) (but see Oka et al., 1984). Such differences in muscle morphology have been found to lead to higher absolute isometric force in male muscles, without a difference in isometric stress (force per cross-sectional area) between sexes in two distantly related species, *Rana catesbeiana* and *Bufo marinus* (Clark and Peters, 2006; Peters and Aulner, 2000). However, these previous studies did not compare muscle mechanics during the reproductive season, but did propose that sexual dimorphism probably increases during the reproductive phase of males. Flexor carpi radialis (FCR) muscles from both male and female *Rana temporaria* had a significantly slower rate of twitch force activation and a slower half twitch relaxation time in the breeding season when compared to outside of the breeding season (Melichna et al., 1972). However, throughout the year sexual dimorphism was evident with higher FCR ATPase activity and faster twitch activation and relaxation rates in females than in males. Resting membrane potential of sternoradialis increased during the breeding season, such that very little stimulation was required to elicit muscle force (Kirby, 1983).

The aims of our study were to use an explosive breeding frog (*Rana temporaria* Linnaeus 1758) during the breeding season to: (1) determine typical male forelimb joint angles used during amplexus; (2) measure *in situ* extensor carpi radialis (ECR) muscle length in male forelimbs held at the typical forelimb joint angles used during amplexus; (3) compare ECR muscle mass and length between male and female frogs; and (4) compare ECR muscle isometric activation times, relaxation times, absolute force, relative force (stress) and fatigue between male and female frogs. For this study we have focused on the ECR, a muscle that plays a fundamental role in amplexus by extending the wrist in a dorsal direction to help prevent the female from moving (Duellman and Trueb, 1994). We propose that the degree of sexual dimorphism in ECR muscle contractile properties will be higher during the breeding season than previously found outside the breeding season (Peters and Aulner, 2000).

Materials and methods

Animals

Male (*N*=7) and female (*N*=7) *Rana temporaria* Linnaeus 1758 were captured from ponds in Coventry and Warwickshire, UK. Frogs were temporarily transferred to a plastic tank of water (0.59 m×0.37 m×0.30 m, length × width × height). On arrival at Coventry University, frogs were transferred to a tank of the same size, each containing 2–5 frogs kept at 16±2°C in a moist soil environment with a small 'pool' of water that contained vegetation. Isolated muscle mechanics experiments were performed 2–8 days after capture.

Amplexus joint angle measurements

The amplexus joint angle measurements were obtained from two couples found amplexing in the field and from two amplexing couples formed in captivity. Pieces of flexible wire were bent into the shapes of the shoulder, elbow and wrist angles and held against the amplexed couple to check the wire shape was representative before further reshaping of the wire or measurement of the angle from the wire. More specifically the amplexus joint angles were measured as follows: the interior angle between the posterior border of the scapula and the posterior humerus (shoulder angle); the interior angle between the humerus and the radioulna (elbow angle); the posteromedial angle between the radioulna and the carpus (wrist angle). Amplexed couples were not disturbed by this process and did not attempt to move.

Morphometrics and dissection

Snout–vent length and maximal diameter of the forelimb (both proximally and distally to the elbow) were measured to the nearest 0.1 mm using Vernier calipers. Frog body mass was measured to the nearest 0.1 g using an electronic balance.

Individuals were killed by pithing and transection of the spinal cord in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Both forelimbs were removed from the frog and pinned out in oxygenated (95% O_2, 5% CO_2) Ringer solution (composition in mmol l^{-1}: NaCl, 115; KCl, 2.5; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; sodium pyruvate, 5.0; $CaCl_2$, 1.8; pH 7.4 at 20°C). The left forelimb was pinned out in the position corresponding to the mean shoulder, elbow and wrist angles observed in amplexus (see Results). The total *in situ* length of the ECR muscle was determined by measuring the length of a piece of suture silk placed along the surface of the muscle and cut to the same length as the muscle. The ECR muscle was dissected, for subsequent muscle mechanics, from the left forelimb with a piece of the humerus left at the end of the proximal tendon, and the thumb left at the end of the distal tendon.

Isometric studies on isolated muscle

The ECR muscle preparation was transferred to a bath of oxygenated (95% O_2, 5% CO_2) circulating frog Ringer solution maintained at 10.0±0.5°C. The humerus was attached *via* a crocodile clip to a strain gauge (model UF1, Pioden Controls Ltd, Canterbury, UK; range ±2 kg for male frogs, ±55 g for

female frogs) and the thumb was attached *via* a crocodile clip to a servomotor (V201, Ling Dynamic Systems, Royston, UK). A linear variable displacement transformer (DFG5.0, Solartron Metrology, Bognor Regis, UK) was used for position detection. The ECR muscle from male frogs was initially set at the length that had occurred *in situ* when the leg was set at the mean joint angles measured during amplexus. Muscle length was measured using a microscope fitted with an eyepiece graticule. The ECR muscle from female frogs was not initially set at any particular length of relevance. Electrical stimulation was delivered to the muscle in 1.5 ms pulses *via* parallel platinum electrodes. A series of isometric twitches were used to optimize stimulation amplitude to yield the maximum isometric twitch force (achieved at a stimulation amplitude between 18 and 20 V). Muscle stimulation was controlled using custom written software (Testpoint, CEC, Massachusetts, USA) *via* a D/A board (KPCI3108, Keithley Instruments, Ohio, USA). Force data were sampled at a rate of 10 kHz. An isometric tetanic response was elicited by subjecting the muscle to a 500 ms train of stimulation. Stimulation frequency was optimized (between 100 and 120 Hz) to yield the maximal isometric tetanic force. The stimulation amplitude and frequency found to generate maximal force for a muscle at *in vivo* amplexus length was then used for all subsequent work with that muscle. A 5 min recovery period was allowed between each tetanic response. Time to half peak twitch, time from peak twitch to half relaxation, time to half peak tetanic force and time from the last stimulus to 50% tetanus relaxation were also measured (the times reported in the Results were measured at the length at which the muscle preparation produced maximal stress). The muscle length was then altered (in no set order) to at least six new lengths to produce a length–force curve *via* repetition of the twitch and tetanus at each new length. At every fourth length change the muscle was set back to the amplexus length (or in the case of female frogs, the first muscle length) so that the condition of the muscle could be monitored over the time course of the experiment. Any changes in muscle force production between these repeated (control) tetanic responses were assumed to have occurred in a linear manner over time and actual muscle force production was corrected accordingly. Usually muscles improved slightly during the time course of the experiment and then decreased to 95% of maximum control force by the end of the experiments. The muscle length for maximum force production (L_0) was recorded.

Fatigue resistance was assessed at *in vivo* amplexus length for males and at the length for maximal force production for females, by subjecting the muscle to a 200 ms train of stimulation (delivered at the stimulation frequency that yielded maximum tetanic force), repeated at a frequency of 1 Hz, for 3 min. Muscle force was monitored during these fatigue tests using a thermal arraycorder. Recovery from the fatigue run was monitored in four ECR muscle preparations that were each subjected to a fatigue run lasting 3 min. Each preparation recovered to greater than 66% of the prefatigue force within 5 min of the fatigue run finishing. Additionally a second fatigue test was delivered 15 min after the end of the first test in one individual. 10 min after this second fatigue test the peak tetanic force was 76% of the peak tetanic force achieved before the first fatigue test. Therefore, we concluded that the fatigue protocol used in this study fatigued rather than killed the muscle preparation.

ECR muscle mass was determined to the nearest 0.1 mg for male frogs (0.01 mg for female frogs) at the end of the experiment using an electronic balance, having first blotted the muscle on absorbent paper to remove excess Ringer solution. Mean muscle cross-sectional area was calculated from mean muscle fibre length, muscle mass and an assumed muscle density of 1060 kg m^{-3} (Méndez and Keys, 1960). Muscle stress was calculated as force divided by mean muscle cross-sectional area. To aid comparisons between sexes and different sized animals muscle force was also expressed as a percentage of body weight (N).

Statistical analysis

To meet the requirements of parametric statistical analyses all percentage data were converted to proportions, then arcsine transformed prior to statistical analysis. Sex comparisons of equality of variance were performed using Levene's test. Independent samples *t*-tests were used to determine whether significant differences existed between sexes in each variable tested, assuming equal variance where appropriate. The effect of fatigue on tetanic force production was analysed using a general linear model to determine the effect of sex on force produced and the interaction between sex and time. The truncated product method (Zaykin et al., 2002) was used to combine each set of *P* values to determine whether it was likely that significant results in the set were truly significant or were due to chance. The summary *P* value from the truncated product method was then stated in each Table legend.

Results
Amplexus joint angles

The male frog shoulder, elbow and wrist angles measured during amplexus were 122±7.24°, 91.0±9.76° and 104±9.13°, respectively (mean ± s.d., *N*=4).

Morphometrics

The female frogs had a significantly (*P*<0.05) longer (7.8%) snout–vent length and tended to have a higher body mass (Table 1). However, the male frogs had significantly (*P*<0.05) larger maximum lower forelimb and upper forelimb diameters than the females, more than 30% higher in each case (Table 1). Male frogs had significantly ECR muscle mass than females (*P*<0.001), comprising means of 0.22% of body mass in males, yet only 0.019% in females (Table 1). Male frogs also had significantly longer (38%) ECR muscles than females (*P*<0.01; Table 1).

Muscle mechanics

The ECR muscle in males produced a significantly higher peak tetanic absolute force and a significantly higher maximal

Table 1. *Morphometric and extensor carpi radialis (ECR) muscle force data for Rana temporaria*

	Male	Female	P
Snout–vent length (mm)	69.5±4.9	75.4±5.0	0.046
Body mass (g)	30.2±7.2	33.9±6.6	0.34
Upper forelimb diameter (mm)	7.63±1.66	5.50±0.42	0.014
Lower forelimb diameter (mm)	9.31±1.47	7.13±0.68	0.004
ECR muscle mass (mg)	65.7±0.9	6.50±2.41	<0.001
ECR muscle length (L_0) (mm)	15.3±3.0	11.1±1.0	0.004
Maximum ECR tetanic force (N)	1.60±0.25	0.101±0.047	<0.001
Maximum ECR tetanic stress (kN m^{-2})	353±79	164±57	<0.001

L_0 is the muscle length for maximum force production.

Values are mean ± s.d. ($N=7$ for each sex). P values are given for independent sample *t*-tests of sex comparison. Truncated product method analysis suggested that the significant results in this table were genuine and unlikely to be due to chance (summary $P<0.001$).

isometric stress (relative force) than in females (Table 1; $P<0.001$ in each case), with values 1500% and 115% higher than female values, respectively. There was no significant difference in time to half peak twitch force between male and female ECR muscles ($P>0.05$; Table 2). However, time from peak twitch force to half relaxation, time to half peak tetanic force and time from last stimulus to half tetanic relaxation were all significantly slower in male ECR muscles being 58%, 74% and 189% longer, respectively, than in female ECR ($P<0.05$ in each case; Table 2). Muscle length had a large effect on force production with maximum force in males produced at 94.4±9.4% of *in vivo* amplexus length.

Male ECR muscle was significantly more fatigue resistant than female ECR (Fig. 1; $P<0.001$ for effect of sex and interaction between time and sex). Peak tetanic force increased in the first few tetani produced by male ECR muscles, then subsequently decreased whereas the peak tetanic force produced by female ECR muscles gradually decreased (Fig. 1). After the first 2–3 s of a fatigue run, male ECR muscles did not relax inbetween trains of stimuli and approached a force plateau. In contrast, female muscle relaxed almost to baseline force inbetween each train of stimuli (Fig. 2).

Discussion

Morphometrics

Male *Rana temporaria* had significantly larger (more than 30%) maximum lower forelimb and upper forelimb diameters than females despite the females having significantly larger snout–vent lengths, i.e. overall male body size was smaller. These findings match the tenfold greater ECR muscle mass and the 1.4-fold greater ECR muscle length in males, when compared with females. Our findings imply that the higher ECR muscle mass in male *Rana temporaria* is largely due to higher muscle cross-sectional area. In sexually dimorphic forelimb muscles, including ECR, fibre cross-sectional areas and fibre number were significantly greater in male than in female *Bufo japonicus* (Oka et al., 1984). Previous studies have also demonstrated sexual dimorphism in mass of forelimb muscles used by males during amplexus (Lee, 2001;
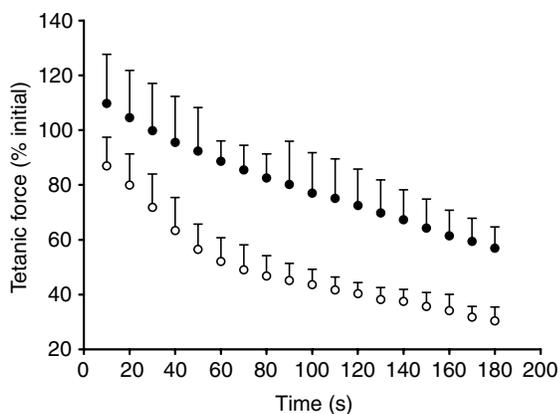

Fig. 1. Changes in peak tetanic force production during fatigue testing of *Rana temporaria* extensor carpi radialis muscle. Values are mean ± s.d. ($N=5$ for males, $N=7$ for females). Males, closed symbols; females, open symbols.
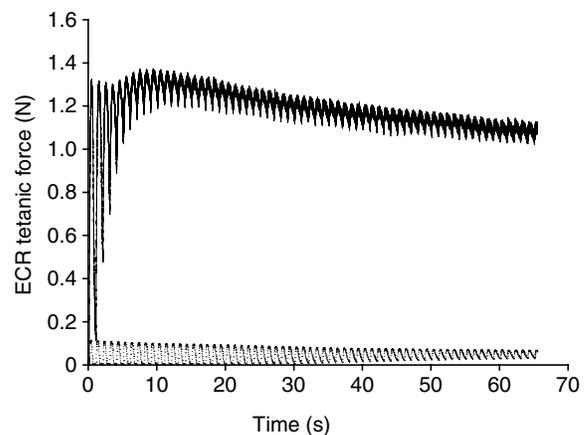
Fig. 2. Typical raw force traces plotted against time for a male (solid line) and a female (broken line) extensor carpi radialis muscle from *Rana temporaria* during fatigue testing.

Table 2. *Rana temporaria extensor carpi radialis muscle twitch and tetanus activation and relaxation times at the length for maximum force production*

	Male	Female	P
Time to half peak twitch force (ms)	28.7±4.79	27.3±6.65	0.65
Time from peak twitch force to half relaxation (ms)	180±51.6	114±27.4	0.011
Time to half peak tetanic force (ms)	68.6±12.2	39.4±12.6	0.001
Time from last stimulus to half tetanic relaxation (ms)	658±118	227±51.7	<0.001

Values are mean ± s.d. (*N*=7 for each sex). *P* values are given for independent sample *t*-tests of sex comparison. Truncated product method analysis suggested that the significant results in this table were genuine and unlikely to be due to chance (summary *P*<0.001).

Oka et al., 1984; Peters and Aulner, 2000; Yekta and Blackburn, 1992). Oka et al. found an approximately sevenfold greater ECR wet muscle mass in male than in female *Bufo japonicus* in breeding state (Oka et al., 1984). In contrast only a 3.4-fold sex difference in ECR muscle mass was found in *Rana pipiens* experimentally induced into breeding state (Yekta and Blackburn, 1992), compared with only a 1.4-fold difference in ECR muscle in *Rana catesbeiana* outside of the breeding season (Peters and Aulner, 2000). Some of these differences in degree of sexual dimorphism between experimental studies may reflect differences between ECR muscles from the natural breeding state compared with experimentally induced breeding state or the non-breeding state. However, differences between species would also be expected because amplexus is a highly diverse aspect of anuran reproductive biology, particularly regarding duration and force requirements. A couple might remain in amplexus for several months, as with some species in the genus *Atelopus,* or for less than an hour, as in *Colostethus*, *Hyla andersonii* and *Pseudacris nigrata* (Wells, 1977). Interspecific differences in sexually dimorphic muscles do appear to exist as four of the muscles that exhibit sex differences in muscle mass in *Rana pipiens* (Yekta and Blackburn, 1992) show no such dimorphism in *Bufo japonicus* (Oka et al., 1984).

Our proposed ECR length for amplexed males was taken as an estimate of the operating conditions of this muscle in nature, although this value would clearly differ according to the size of female the male was copulating with. ECR produced maximal force at lengths compatible with those we estimated for amplexed males, suggesting that *in vivo* operating conditions during the breeding season might generally be close to the functional optima of the muscle. In contrast, ECR out of breeding season produced maximal force at 120% of their estimated amplexus length (Peters and Aulner, 2000). The significance of this difference is difficult to evaluate at this point because we lack information on the main use of this muscle during the non-breeding season, force–length relationships might differ between Ranid species, and male *Rana temporaria* appear not to choose females according to body length, body mass or fecundity (Elmberg, 1991), so that amplexed *Rana temporaria* males in nature might experience a relatively wide range of *in vivo* muscle lengths.

Muscle mechanics

Male *Rana temporaria* ECR muscle produced a 25.8-fold significantly higher isometric absolute tetanic force and a 2.2-fold significantly higher relative tetanic force (stress) than in females. It appears that most of the difference in absolute force between sexes is due to the large increase in cross-sectional area with a relatively small contribution from the increase in stress. Peters and Aulner also found higher (1.09-fold higher) ECR muscle absolute forces in males than females (Peters and Aulner, 2000); however, they found no significant differences between sexes in muscle stress (relative force), indeed muscle stress tended to be higher in female ECR. They reported values of 237 kN m^{-2} for maximal isometric stress and an overall force production equal to 150% of body weight in ECR from male non-breeding state *Rana catesbeiana* (Peters and Aulner, 2000). In contrast, our corresponding values for male *Rana temporaria* at peak reproductive season were 381 kN m^{-2} and 560%, respectively. Undoubtedly, intraspecific comparisons would be more appropriate to enable conclusive statements; however, given the magnitude of the differences found and previous morphological studies, we suggest that the force production of male forelimb muscles responsible for amplexus is probably much higher when frogs are in reproductive state, and that such changes might be hormonally induced causing increased muscle mass, although we have no evidence to exclude some of the change being due to increased muscle stress. However, protein concentration in seven different forelimb muscles has not previously been found to differ between male and female *Rana pipiens* that have been experimentally induced into breeding state (Yekta and Blackburn, 1992).

We found that ECR relaxation times were significantly longer in males than in females. This slower relaxation in males led to very limited relaxation (sustained force) between tetani during the fatigue run, such that the male ECR muscle could maintain almost constant isometric force when stimulated for only one fifth of the time (for 200 ms in every 1 s). Previous studies on anuran dimorphic forearm muscles outside of the reproductive season have also reported a higher sustained force in males than in females (Clark and Peters, 2006; Peters and Aulner, 2000), but nothing like as high as we have shown in frogs of reproductive state. The twitch half relaxation time of flexor carpi radialis muscle in *Rana*

temporaria was 94% longer (slower) in males than in females during the breeding season and such large differences persisted throughout the year, with muscle in males possessing more fibres with low myosin ATPase activity (Melichna et al., 1972). Peters and Aulner found ECR twitch relaxation times were 52% slower in male than in female *Rana catesbeiana* outside of the reproductive season, although these values did not reach the level of significance (Peters and Aulner, 2000). However, twitch relaxation times were significantly slower in male than in female abductor indicus longus (AIL) and flexor carpi radialis (FCR) muscle (Peters and Aulner, 2000). Similar (although non-significant) trends in twitch relaxation times, but significantly slower tetanus relaxation times, in male than in female AIL and FCR in *Bufo marinus* outside of the reproductive season were also found (Clark and Peters, 2006). Peters and Aulner (Peters and Aulner, 2000) found much faster ECR relaxation times in male *Rana catesbeiana* than we found in male *Rana temporaria* but these differences may again reflect differences in reproductive state between the two studies; indeed the contractile properties of flexor carpi radialis muscle tend to become faster outside of the breeding season (Melichna et al., 1972). In the present study the rate of fatigue of male ECR muscle was relatively slow compared to female ECR muscle. In contrast there was no significant difference in fatigue index between sex in ECR muscle of *Rana catesbeiana*, studied during the non-reproductive phase (Peters and Aulner, 2000). Seasonal changes in contractile properties are likely initiated by hormonal changes. Testosterone treatment of castrated *Xenopus laevis* increased tetanus half relaxation time by 38% and increased both tetanic force and muscle cross-sectional area by more than 80% in flexor carpi radialis muscle when compared with castrated control males (Regnier and Herrera, 1993). Changes in muscle fiber type proportion can also be induced by testosterone, as has been reported for *Xenopus laevis* (Rubinstein et al., 1983) and suggested for *Rana pipiens* (Renaud, 1991).

Application to reproductive behaviour

Our results suggest that the extensor carpi radialis (ECR) muscles from male *Rana temporaria* in breeding state are suited to maintaining low-to-moderate forces for long periods of time, but are also able to produce very high peak forces if required. These physiological properties match the two different clasping behaviours previously described for *Xenopus laevis*: "*(i) a prolonged but loose maintenance grip and (ii) brief rapid tightening*" (Regnier and Herrera, 1993). The results from the present study, combined with evidence from previous studies, suggest that extensor carpi radialis in Ranid species undergoes large increases in mass, relaxation time and fatigue resistance as males enter their reproductive state, enabling the forelimbs to produce prolonged relatively high force to clasp (hold) females during amplexus. These characteristics agree with what is known about the reproductive biology of *Rana temporaria*. At low male densities, the reproductive mode of *Rana temporaria* is

characterized by random mating, with no clear size ratio between males and females and an absence of takeover events (Arak, 1983; Elmberg, 1987). Non-random mating, however, occurs at high male densities and possibly also when the prespawning period is relatively long. Under experimental conditions involving high male densities and high male to female ratios, takeover attempts become common and are successful in 20% of couples (Elmberg, 1991). Therefore, takeovers, even if rare, might have modulated the evolution of forelimb muscle traits in this species. High strength of the forelimbs might offer selective advantages enabling males to better prevent both interference from non-amplexed males and, to a lesser extent, female rejection. Our understanding of anuran sexual selection and reproductive success would benefit from additional comparative studies of the physiology and ecological correlates of forelimb muscles.

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