

Reproductive roles predict sexual dimorphism in internal and external morphology of lake whitefish, *Coregonus clupeaformis*

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Abstract – The different reproductive roles of the sexes can predict the direction and magnitude of sexual dimorphism of external and internal morphology. Males should have enlarged structures that enhance the acquisition of mating opportunities, whereas females are predicted to have enlarged organs that are associated with the production of eggs. We tested these predictions in male and female lake whitefish, a species in which both sexes have similar overall body size and shape. After controlling for body size, male lake whitefish had significantly longer jaws and pectoral and pelvic fins, larger hearts, and more muscle than females. Sexual dimorphism in relative muscle mass may be one of the most fundamental morphological differences between males and females. Females had relatively heavier livers than males. Because the liver is important for the breakdown of fats and vitellogenesis, selection should favour an enlarged liver in females for the processing of energy and the production of large numbers of eggs.

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Un resumen en español se incluye detrás del texto principal de este artículo.

Introduction

By definition, males and females differ in their reproductive roles. In most animals, males maximise their reproductive success by seeking, guarding and mating with multiple females (Trivers 1972). Females benefit from producing eggs and expending energy to maximise survival of their offspring (Trivers 1972). Differences in the selective pressures experienced by the sexes can ultimately result in the evolution of sexual dimorphism of morphological traits (Andersson 1994). Thus, the magnitude and direction of sexual dimorphism of both external and internal characters can be predicted by examining the reproductive roles of the sexes within a species.

Sexual dimorphism in overall size and appearance occurs throughout the animal kingdom (Hedrick & Temeles 1989) and has thus far dominated research on sexual differences in morphology (e.g. Weatherhead

et al. 1995; Badyaev & Martin 2000; Schulte-Hostedde et al. 2002). However, other, less obvious, morphological traits are also predicted to be sexually dimorphic. Subtle sex differences may occur in external traits such as fin size of fish because males with large fins tend to be more successful at mate acquisition and male–male competition due to increased speed and manoeuvrability (Fleming & Gross 1994; Kokita & Mizota 2002).

In addition to external characters, sexual selection should also result in sexual dimorphism of internal organs. For example, females tend to have larger gonads than males with large, energy rich eggs, whereas males have much smaller gonads that produce numerous, relatively inexpensive sperm (Andersson 1994). However, there has been little investigation examining differences in other internal body traits.

The reproductive roles of males predict that males should have enlarged organs that increase the

likelihood of mating successfully. Presumably, selection should favour a robust musculature which would aid in locomotion, mate acquisition and male–male competition. Indeed, sex differences in relative muscle mass are likely to be the most fundamental differences between the sexes across the animal kingdom (Bonnet et al. 1998). In taxa as diverse as snakes and small mammals, males have relatively more muscle than females (Bonnet et al. 1998; Schulte-Hostedde et al. 2001). Additionally, males are expected to have larger hearts to supply the increased musculature with blood and thus increase aerobic capacity. Female reproductive roles predict that females should have larger organs associated with food acquisition and processing to support the production of energy-rich eggs. The intestines and liver are critical for the absorption of nutrients and the processing of fats, respectively (Eckert et al. 1996), and thus females should have larger intestines and larger livers than males. The only study that has tested this prediction found that female snakes tend to have larger viscera and livers than male snakes; however, this relationship was not found in all snake species examined (Bonnet et al. 1998).

The adaptive interpretation of sexual dimorphism in external and internal traits may be compromised by sex differences in body shape or overall size. It is therefore critical to test predictions related to sex differences in morphology using species in which males and females do not differ in overall structural size. In cases where there is substantial overlap in body size, any morphological differences found between the sexes cannot be easily attributed to allometry or differences in shape. Mature male and female lake whitefish (*Coregonus clupeaformis*) have similar body sizes and shapes (Scott & Crossman 1973) and thus are an excellent species with which to examine sexual dimorphism in internal and external characters.

Little is known about the mating system of whitefish, but it has been described as one in which males engage in both overt competition and sperm competition. For example, Becker (1983) describes whitefish spawning as involving one or two males interacting with a single female with males simultaneously spawning the female as she rises to the surface. It has also been suggested that *Coregonus* spp. engage in group-spawning behaviour (Wedekind et al. 2001), a situation in which sperm competition might take place. The presence of breeding tubercles (Becker 1983), particularly on males, indicate that other tactile cues may be associated with female mate choice and the mating system dynamics of this species (Kratt & Smith 1978). The potential for sperm competition does not negate the importance of male–male competition. Individual whitefish sperm swim for a short period (<1 s) and travel a very short distance (G. Burness &

R. Montgomerie, unpublished data) and so placement relative to the female during spawning must be very important. The huge differences in relative gonad investment between the sexes (T. Johnston, personal communication) also indicate that structures associated with male–male competition and mate acquisition are predicted to be relatively larger in males, and structures associated with food acquisition and energy processing should be relatively larger in females.

Methods

Lake whitefish were collected using trap nets from Bay of Quinte, Lake Ontario (44°N, 77°W) prior to spawning in October 2002. Collection was limited to adult fish in spawning condition to avoid variation caused by maturity status. A total of 40 lake whitefish (20 females, 20 males) were sampled. Prior to processing the fish were stored on ice. All fish were processed within 24 h of being collected.

External morphology

For each fish, measurements (± 1 mm) were taken of total length, fork length, jaw width and length, right and left pectoral fin and pelvic fin length, caudal fin length, anal fin length, dorsal fin height and total body mass (± 0.1 g). Jaw width was defined as the maximum internal width of the open mouth, and jaw length was measured with the mouth closed from the tip of the snout to the anterior end of the maxillary bone. All fin measurements with the exception of caudal fin length were taken from the base of the fin to its maximum length or height. Due to difficulty in determining the base of the caudal fin, caudal fin length was taken as the distance between the posterior base of the anal fin and the tip of the ventral fork.

Internal morphology

Internal body components were carefully dissected and weighed (wet masses, ± 0.01 g). Intestine, liver, heart, visceral fat and muscle mass were measured. For intestine mass all contents were removed prior to weighing. Muscle mass was measured as the eviscerated carcass mass minus head and tail but including bone and skin (both of which should be negligible).

All mass and size variables were log (base 10) transformed to linearise all relationships. The value of a particular fin length entered into the analysis was taken as the mean of the right and left sides. Analysis of covariance (ANCOVA) was used to compare the sexes for a given trait, after controlling for body size. Fork length was used as the covariate (body size) in the analysis rather than total length to avoid the confounding effects of differences in caudal fin

Table 1. Raw mean \pm SE and least squares mean (in parentheses) of external morphological traits for male ($N = 20$) and female ($N = 20$) lake whitefish, and results of analyses of covariance on sex differences in relative size (using fork length as continuous predictor) of external traits

	Male	Female	$F_{1,37}$	P
Fork length (mm)	462.8 \pm 17	463.9 \pm 15	–	–
Total mass (g)	1093.1 \pm 32.5 (1091.4)	1211.0 \pm 35.2 (1193.6)	11.66	0.002*
Somatic mass (g)	1075.9 \pm 31.9 (1073.91)	988.8 \pm 25.3 (976.8)	16.74	<0.001*
Jaw width (mm)	20.4 \pm 0.3 (20.4)	19.7 \pm 0.4 (19.5)	3.47	0.070
Jaw length (mm)	25.2 \pm 0.3 (25.2)	24.3 \pm 0.4 (24.1)	4.94	0.032
Caudal fin length (mm)	135.9 \pm 1.4 (135.9)	135.2 \pm 1.2 (134.9)	0.39	0.534
Pectoral fin length (mm)	72.6 \pm 0.9 (71.7)	69.5 \pm 0.8 (69.3)	6.17	0.018
Pelvic fin length (mm)	62.3 \pm 0.9 (62.3)	60.4 \pm 0.6 (60.2)	4.46	0.042
Anal fin length (mm)	51.5 \pm 1.1 (51.4)	50.3 \pm 0.8 (50.0)	2.00	0.165
Dorsal fin height (mm)	60.5 \pm 0.9 (60.4)	59.3 \pm 0.7 (59.2)	1.02	0.319

Differences between the sexes for traits marked with * remained significant after Bonferroni correction ($\alpha = 0.05/9 = 0.006$).

morphology between the sexes. Bonferroni correction was applied to both sets of analyses (external and internal traits) independently (Rice 1989).

Results

The mean external morphological measurements for male and female lake whitefish are shown in Table 1. Males and females did not differ with respect to fork length ($t = 0.4$, $P = 0.69$, d.f. = 38) but females were heavier than males ($t = 2.49$, $P = 0.02$, d.f. = 38) even when controlling for fork length (Table 1). Males had more somatic mass (total mass minus reproductive organs) than females, after controlling for fork length (Table 1). Despite these significant differences, there was considerable overlap in somatic mass between the sexes (males: 870–1350 g, females: 832–1265 g). Analyses of covariance showed that, after controlling for fork length, males had longer jaws and longer pectoral and pelvic fins than females, although these differences are rendered nonsignificant after Bonferroni correction (Table 1). Slopes for male versus female external morphology were homogeneous for all measurements ($F < 1.3$, $P > 0.26$).

Table 2 presents the mean weights of internal traits for male and female lake whitefish. When corrected for body size, males and females differed with respect to the mass of liver, heart, visceral fat and muscle (Table 2, Fig. 1). Males had larger hearts and more muscle and visceral fat than females, and females had

larger livers than males. Females also tended to have larger intestines, after controlling for body size ($P = 0.07$). In all analyses of covariance in which there were significant differences between males and females the slopes were homogeneous ($F < 1.3$, $P > 0.3$).

Discussion

The differences in the reproductive roles occupied by the sexes should influence patterns of selection and thus should ultimately lead to sex differences in morphology. Males should have adaptations that increase the probability of acquiring mates and of success at male–male competition. Females should be under selection to acquire, process and store energy to facilitate the production of offspring. In mature male and female lake whitefish, sexual dimorphism of both external and internal morphology was consistent with these predictions. Male lake whitefish had enlarged characters which would be expected to be beneficial in male–male competition and mate acquisition. Female lake whitefish had enlarged organs that are associated with the processing and absorption of energy. A caveat to this explanation is that ecological factors such as sex differences in diet can affect the evolution of sexual dimorphism (Shine 1989). There is little evidence that male and female lake whitefish differ with respect to diet (e.g. Becker 1983) and so we assume that differences in reproductive roles have led to our results.

Table 2. Raw mean \pm SE and least squares mean (in parentheses) of internal traits for male ($N = 20$) and female ($N = 20$) lake whitefish, and results of analyses of covariance on sex differences in relative size (using fork length as continuous predictor) of internal traits

	Male	Female	$F_{1,37}$	P
Intestine (g)	36.83 \pm 1.64 (34.20)	40.94 \pm 1.59 (37.16)	3.48	0.070
Liver (g)	9.16 \pm 0.39 (9.05)	13.39 \pm 0.73 (13.99)	33.65	<0.001*
Heart (g)	1.38 \pm 0.07 (1.35)	1.22 \pm 0.06 (1.19)	5.02	0.031
Visceral fat (g)	1.50 \pm 0.33 (2.18)	0.91 \pm 0.72 (1.32)	7.87	0.008*
Muscle (g)	823.17 \pm 25.69 (821.14)	737.66 \pm 19.49 (728.18)	23.77	<0.001*

Differences between the sexes for traits marked with * remained significant after Bonferroni correction ($\alpha = 0.05/5 = 0.01$).

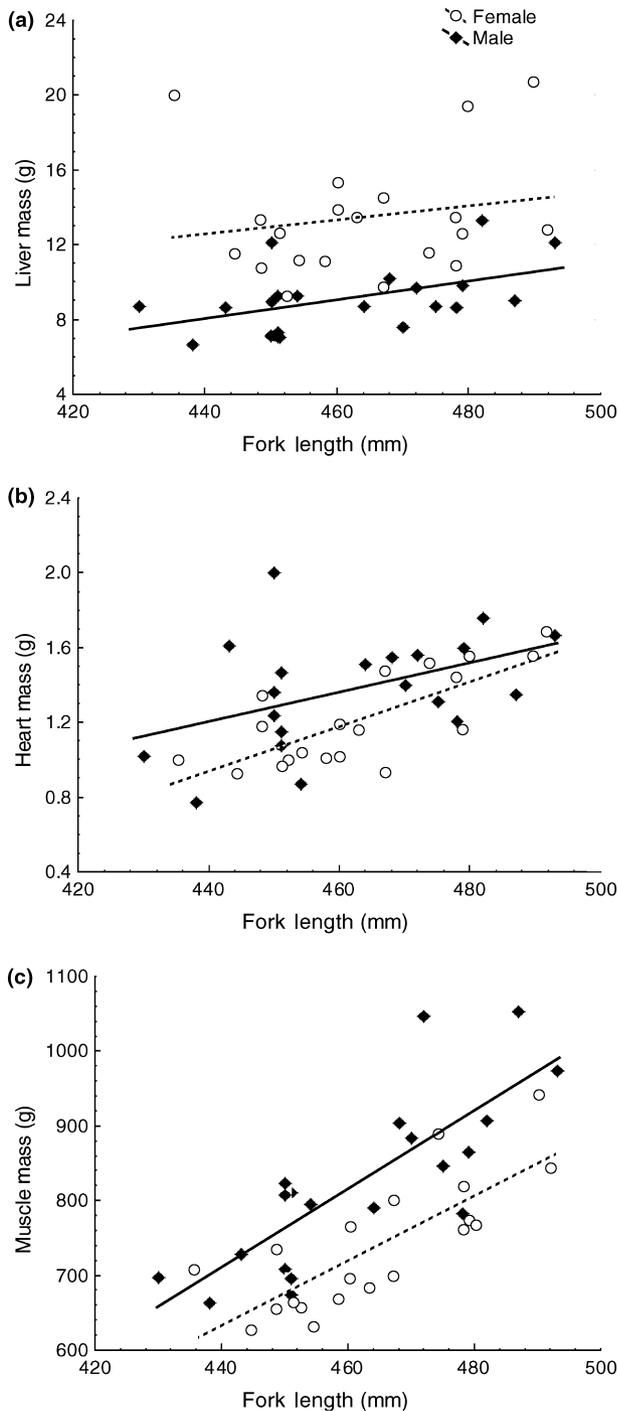


Fig. 1. Sexual dimorphism of internal traits [liver mass (a), heart mass (b) and muscle mass (c)] relative to body size in lake whitefish. Raw data are presented along with the best-fit regression lines. Plots for raw data show the same patterns as for log-transformed data. Controlling for body size, females have greater liver mass while males have greater heart mass and muscle mass.

Perhaps the most interesting results from this study are the sex differences in the relative size of internal traits. The relatively large muscle mass and heart of male whitefish should be important for increased

swimming speed (Videler 1993) and manoeuvrability for male–male competition and mate acquisition. This is consistent with earlier suggestions that male-biased dimorphism with respect to muscle mass may be a fundamental difference between the sexes that is consistent across the animal kingdom (Bonnet et al. 1998; Schulte-Hostedde et al. 2001). Relatively large hearts supply the muscle mass with oxygenated blood, and heart mass is related to swimming performance in winter-acclimatised largemouth bass (*Micropterus salmoides*) (Kolok 1992). Additionally, individuals with increased red muscle activity had superior swimming performance (Kolok 1992). There is evidence from rainbow trout (*Oncorhynchus mykiss*) that sex differences in muscle mass and heart mass may be the result of effects of hormones such as 11-ketotestosterone on these traits (Thorarensen et al. 1996). This suggests the intriguing possibility that some sex differences in internal organ size are the result of phenotypic flexibility (Piersma & Drent 2003) rather than ontogenetic differences.

The liver of female whitefish is important for food processing and contributes to the production of energy-rich eggs (Marshall et al. 1999). The liver produces vitellogenin which is further processed into yolk proteins, and larger livers are associated with higher production of vitellogenin and higher energy content (Lambert & Dutil 1997; Dahle et al. 2003; Guijarro et al. 2003). In other species of fish the liver participates in transferring lipids from the viscera to the ovary for egg production and development (Henderson et al. 1996). Indeed, the importance of the liver for the production of bile and the digestion of fats for egg production may be a characteristic common to fish and other vertebrates. In both Atlantic silverside (*Menidia menidia*) and inland silverside (*Menidia beryllina*), females have larger livers than males (Huber & Bengtson 1999). Similar patterns have been found in house sparrows (Chappell et al. 1999) and the European asp (*Vipera aspis*) (Bonnet et al. 1998).

Female intestines were larger than the intestines of males (but not at the $\alpha = 0.05$ level). However, it is unclear whether this reflects ontogenetic differences in the sexes, or changes in gut morphology caused by differences in diet associated with reproduction (Green & Millar 1987). The observed pattern in which reproductively active females had larger intestines than males has been found in several small mammal species (Norrie & Millar 1990), indicating some generality in this pattern.

Trends towards male-biased sexual dimorphism in jaw length, and pectoral and pelvic fin length likely result from selection for enlarged male characters associated with male–male competition and mate acquisition. In male coho salmon (*Oncorhynchus*

kisutch), for example, large jaws have been identified as a specialised weapon used during breeding competition (Fleming & Gross 1994). Male coho salmon with relatively larger jaws attended more females, attained more spawnings and had greater overall breeding success (Fleming & Gross 1994). The increased agility and combative capability resulting from larger pectoral and pelvic fins in males should also aid in competition with other males for access to mates. Larger fins are beneficial when male–male competition occurs, as is observed in other closely related salmonid species (Pettersson et al. 1999; MacLean et al. 2000). Additionally, larger fins should aid in stability and control of a swimming fish (Blake 1983; Videler 1993) and may help males position themselves optimally relative to females during spawning in order to maximise fertilisation success. Sperm from lake whitefish are motile for a short duration (<1 min; G. Burness and R. Montgomerie, unpublished data), thus male positioning during spawning must be critical for fertilisation success and as a result is an important determinant of reproductive success.

The finding that males had relatively more visceral fat than females is initially surprising considering that females would be expected to have greater energy reserves for the production of eggs. However, because the fish examined were captured just before spawning and had fully developed gonads, females had already mobilised their energy reserves to produce their eggs. If visceral fat had been measured prior to gonadal development, females may have had more visceral fat than males. The finding that males have more visceral fat prior to spawning than females suggests that gonadal development is more costly for females than males. This is in agreement with the initial predictions resulting from the different reproductive roles of males and females. To further examine this utilisation of energy it would be interesting to compare the amount of visceral fat in males and females after spawning to compare energetic costs during spawning.

This study has focused on ultimate explanations of the observed sexual dimorphism, yet it would be interesting to further examine the proximate causes of these differences. For example, are sex differences in organ size the result of phenotypic elasticity manifested through seasonal changes in hormones such as testosterone, or are these differences set from an early age? In addition, determining the sex-specific fitness consequences of variation in internal dimorphism by answering questions such as whether males with larger hearts and more muscle mass have higher mating success or whether females with larger livers produce more or higher quality eggs will further our understanding of the selective pressures that underlie sexual dimorphism.

Resumen

1. Los diferentes papeles reproductivos de los sexos pueden predecir la dirección y magnitud del dimorfismo sexual en la morfología externa e interna. Los machos deberían tener estructuras mayores que estimulen la adquisición de pareja mientras que las hembras deberían tener órganos mayores asociados a la producción de óvulos. Examinamos estas predicciones en machos y hembras de *Coregonus clupeaformis*, una especie en la que ambos sexos tienen formas y tamaños similares.
2. Los machos exhibieron mandíbulas y aletas pélvicas y pectorales significativamente más largas, corazones más grandes y más músculo que las hembras. Dimorfismo sexual en la masa muscular relativa puede ser una de las diferencias morfológicas fundamentales entre machos y hembras. Las hembras tuvieron hígados más pesados que los machos. Dado que el hígado es importante para el metabolismo de las grasas y para la vitelogénesis, la selección debería favorecer mayores hígados en las hembras para procesar la energía y para la producción de mayores números de óvulos.

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