bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Idea Paper: Effects of gonad type and body mass on the time required for sex change in fishes

Soma Tokunaga[1,2], Tatsuru Kadota[3], Yuuki Y. Watanabe[2,4], Tetsuo Kuwamura[5,6] and Yuuki Kawabata[1,7*]

[1]Faculty of Fisheries, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan

[2]Department of Polar Science, SOKENDAI (The Graduate University for Advanced Studies), Tachikawa, Tokyo 190-8518, Japan

[3]Nagasaki Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Taira-machi, Nagasaki, Nagasaki 851-2213, Japan

[4]National Institute of Polar Research, Tachikawa, Tokyo 190-8518, Japan;

[5]School of International Liberal Studies, Chukyo University, Nagoya 466-8666, Japan

[6]Institute for Research in Social Science, Advanced Collaborative Research Organization of Chukyo University, Nagoya 466-8666, Japan

[7]Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan

*Corresponding author: yuuki-k@nagasaki-u.ac.jp

KEYWORDS

individual, experiment, ideas for fundamental questions, scaling, sequential hermaphrodites

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Abstract:

Sex change is a well-known phenomenon in teleost fishes, and it takes several days to a few months depending on the species and direction of sex change. However, the underlying factors influencing the time required for sex change (T_S) remain unclear. Given that the time for producing a new gonad largely determines T_S, the gonad type (i.e., whether fish retain the gonad of opposite sex or not [delimited or non-delimited]) and metabolic rate are the ultimate determinants of T_S. This study sought to test two hypotheses: (1) the delimited gonad shortens T_S; and (2) T_S scales with mass$^{0.1–0.2}$, because the metabolic scaling exponent (β) in fishes is 0.8–0.9 and biological times scale with mass$^{1-\beta}$ in general. We compiled data on T_S for 12 female-to-male and 14 male-to-female sex-changing species from the literature. Results of individual examinations of the effects of gonad type and mass were consistent with our hypotheses. However, upon simultaneous examination of the effects of gonad type and mass, these effects became unclear because of their strong multicollinearity. The compiled data for delimited and non-delimited gonads were biased toward the smaller and larger species, respectively, precluding us from being able to statistically distinguish between these effects. Small species with non-delimited gonads and large species with delimited gonads exist; however, their T_S has not been measured with high temporal resolution thus far. Therefore, additional experiments on these species are required to statistically distinguish between, as well as to better understand, the effects of gonad type and mass on T_S.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

RESEARCH QUESTION

Sex change is a well-known phenomenon in plants and animals (Policansky 1982). Female-to-male and male-to-female sex changes have been observed in teleost fishes (Kuwamura et al. 2020). Sex change may span over several days to a few months, depending on the species and direction of sex change. However, the underlying factors impacting the time required for sex change (T_S) remain unclear. Given that the time required to produce a new gonad largely determines T_S, it is anticipated that there are two factors that may influence T_S. First, some sex-changing species retain the gonad of opposite sex with the "delimited" type, where ovarian and testis tissues are separated by a thin cellular wall. The delimited gonad type is considered an adaptation to reduce the amount of new gonads to produce and shorten the T_S (Munday et al. 2010); however, no such quantitative assessment has been conducted thus far. Second, many biological times, such as longevity and gestation periods, are determined by the metabolic rate of animals, which is a function of temperature and body mass (Gillooly et al. 2001, Brown et al. 2004). Consistent with this theory, warmer water causes shorter T_S in sex-changing fish (Black et al. 2005); however, the effect of body mass on T_S requires clarification. As such, this study sought to address two research questions: (1) whether the delimited gonad shortens T_S; and (2) whether body mass affects T_S.

VALUE

The ultimate rationale for sex change is to increase fitness (Warner 1975). Sex change does not occur

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

instantly (i.e., it takes several days to a few months), and fish lose breeding opportunities during this period. Under specific ecological and physiological constraints, each species is expected to have evolved to minimize T_S. Identifying the factors that determine Ts is essential to understand the decision-making process and life history strategies of sex-changing fish. Additionally, identifying such factors affecting T_S would allow researchers to estimate the T_S of various sex-changing species. Such information is useful to induce sex change in commercially important species and enhance seed production in aquaculture (Quinitio et al. 1997, Sato et al. 2018). This information is also essential to estimate the impact of sex-biased harvesting on the reproductive output of wild populations (Sato et al. 2018).

RELEVANT HYPOTHESIS

This study sought to test two key hypotheses. The first hypothesis is that the T_S of species with delimited gonads is shorter than that of non-delimited gonads. The second hypothesis is that body mass affects T_S through its effect on metabolic rate. More specifically, the metabolic rate Y may be expressed by body mass, M, as $Y \propto M^{\beta}$, where β is a metabolic scaling exponent. Biological times, t_B, such as gestation period, life-span, and population doubling time, were expressed as M/Y, that is, $t_B \propto M^{1-\beta}$. As β is approximately 0.8–0.9 in fishes (White et al. 2007), we hypothesized that T_S scales with $M^{0.1–0.2}$.

From these two hypotheses, we predicted four distinct scenarios. First, if gonad type and

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

body mass determine T_S, the T_S of delimited species is shorter than that of non-delimited species, and the slope in each gonad type is between 0.1 and 0.2 (Figure 1a). Second, if the gonad type alone determines T_S, the T_S of delimited species is shorter than that of non-delimited species, and the slope in each gonad type is almost zero (Figure 1b). Third, if body mass alone determines T_S, the regression slope is between 0.1 and 0.2, and there is no difference in *Ts* between delimited and non-delimited species (Figure 1c). Fourth, if neither factor affects T_S, the slope in each gonad type is almost zero, and there is no difference in *Ts* between delimited and non-delimited species (Figure 1d).

NEW RESEARCH IDEA

To test these hypotheses, we compiled data on T_S (in days) for as many fish species as possible from the literature. *Ts* was measured as the time between the start of the experiment (e.g., removal of the dominant male, introduction of multiple females into a tank) and when fertilized eggs were first observed. We accepted data from laboratory experiments and the field experiments in which fish were monitored daily. As we are interested in the time from when the physiological switch is turned on and when sex change is complete, we subtracted the period of male–male competition from total periods for the male-to-female sex change. For the female-to-male sex change, the number of females in the mating group may affect the time before the physiological switch is turned on through the change in sex hormone concentrations (Yamaguchi 2016). As such, we excluded experiments where only two females were introduced as a mating group (i.e., all monogamous species and some

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

haremic species), and selected experiments in which the number of females was maximum, when calculating the T_S for each species. We did not model temperature effects because water temperatures were not reported in some studies, and because, for studies reporting water temperatures, their variation among sex-changing species was small (~25 °C).

All literature compiled in this study reported fish body size as body length. Therefore, we estimated body mass from total length (TL) using Bayesian length-weight relationships (Froese et al. 2014), with the parameters reported in FishBase (Froese & Pauly 2021). Fish size reported as the standard length (SL) or folk length (FL) was converted to TL based on photos available in FishBase or Nakabo (2018). When the size of sex-changing individuals was not reported in the literature, we used the mean length of experimental fish. If fish size was not reported in the literature, we used the size of mature individuals reported in a different study.

To account for the phylogenetic non-independence of species, we first used the phylogenetic generalized least squares (PGLS) method to examine the effects of gonad type and body mass on T_S (see Appendix S1 for details). However, Pagel's λ was zero in all models, implying that these traits are independent of the given phylogeny, making the ordinary least squares (OLS) method more appropriate (Freckleton et al. 2002). As such, we used the OLS method for subsequent analyses. First, we separately examined the effects of the gonad type and body mass on T_S. Thereafter, we examined the concurrent effects of gonad type and body mass on T_S using multiple regression analysis. Note that T_S and body mass were $\log_{10}$-transformed in all the analyses.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

We compiled data on the T_S of 12 and 14 species for female-to-male and male-to-female sex changes, respectively (Table S1). When the effects of gonad type and body mass were examined separately, the results of both were consistent with our hypotheses. The T_S of the species with delimited gonads was shorter than that of non-delimited gonads in both sex-changing directions (female-to-male: $t = 2.78$, $d.f. = 10$, $p = 0.02$; male-to-female: $t = 2.99$, $d.f. = 12$, $p = 0.01$). The scaling exponent of *Ts* for female-to-male sex change was 0.12 ($t = 2.51$, $d.f. = 10$, $p = 0.03$), which was within the range predicted from the metabolic rate (0.1–0.2) (Figure 2a). Although the exponent of *Ts* for male-to-female sex change was slightly larger than the predicted range (slope = 0.32, $t = 2.46$, $d.f. = 12$, $p = 0.03$), the 95% confidence interval (CI; 0.04–0.60) included the predicted values (0.1–0.2) (Figure 2b).

When the effects of gonad type and mass were examined concurrently, they became unclear in both sex-changing directions. When gonad type was first inputted into the model, the effect of this parameter was significant and the body mass effect was insignificant; however, when body mass was first inputted into the model, the effect of this parameter was significant and the gonad type effect was insignificant (Table 1). These results may be attributable to the strong multicollinearity between gonad type and body mass; the compiled data for delimited and non-delimited gonads were biased toward the smaller (female-to-male, 0.18–0.53 g; male-to-female, 0.13–1.2 g) and larger (female-to-male, 2.8–2561 g; male-to-female, 0.55–15 g) species, respectively (Figure 2; Table S1). This bias precludes the ability to statistically distinguish the effects of gonad type and body mass.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Therefore, additional sex-change experiments are required to fill these data gaps (i.e., small species with non-delimited gonads and/or large species with delimited gonads) and to help better understand how *Ts* is determined in fishes.

HOW TO TACKLE THE QUESTION THROUGH THE PROPOSED NEW IDEA

While some gobiid species (e.g., genera *Coryphopterus*, *Fusigobius*, *Gobiodon* and *Paragobiodon*) have non-delimited gonads and small body sizes (Munday et al. 2010, Kuwamura et al. 2020), some sparid species (e.g., genera *Acanthopagrus*, *Pagrus*, and *Sparus*) have delimited gonads and large body sizes (Buxton & Garratt 1990, Cody & Bortone 1992); their sex change may be observed in large tanks (Kuwamura et al. 2020). Therefore, we propose additional sex-change experiments for these species.

MOTIVATION

There is a need for additional sex-change experiments, specifically on small species with non-delimited gonads and large species with delimited gonads. Typically, sex-change experiments require specialized techniques that are specific to each species. Thus, we believe that sharing our ideas and data through this "Idea Paper" will guide skilled researchers to focus on the *Ts* of these species.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

REFERENCES

Black, M. P., B. Moore, A. V. M. Canario, D. Ford, R. H. Reavis, & M. S. Grober. (2005). Reproduction in context: field testing a laboratory model of socially controlled sex change in *Lythrypnus dalli* (Gilbert). *Journal of Experimental Marine Biology and Ecology*, *318*, 127-143. https://doi.org/10.1016/j.jembe.2004.12.015

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, & G. B. West. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*, 1771-1789. https://doi.org/10.1890/03-9000

Buxton, C. D., & P. A. Garratt. (1990). Alternative reproductive styles in seabrearns (Pisces: Sparidae). *Alternative life-history styles of fishes*, 113-124. https://doi.org/10.1007/978-94-009-2065-1_7

Cody, R. P., & S. A. Bortone. (1992). An investigation of the reproductive mode of the pinfish, *Lagodon rhomboïdes* Linnaeus (Osteichthyes: Sparidae). *Gulf of Mexico Science*, *12*, 3. https://doi.org/10.18785/negs.1202.03

Freckleton, R. P., P. H. Harvey, & M. Pagel. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist*, *160*, 712-726. https://doi.org/10.1086/343873

Froese, R., & D. Pauly. (2021). FishBase. World Wide Web electronic publication. Version (02/2021). Retrieved from www.fishbase.org.

Froese, R., J. T. Thorson, & R. Reyes Jr. (2014). A Bayesian approach for estimating length-weight relationships in fishes. *Journal of Applied Ichthyology*, *30*, 78-85. https://doi.org/10.1111/jai.12299

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, & E. L. Charnov. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*, 2248-2251. https://doi.org/10.1126/science.1061967

Kuwamura, T., T. Sunobe, Y. Sakai, T. Kadota, & K. Sawada. (2020). Hermaphroditism in fishes: an annotated list of species, phylogeny, and mating system. *Ichthyological Research*, *67*, 341-360. https://doi.org/10.1007/s10228-020-00754-6

Munday, P. L., T. Kuwamura, & F. J. Kroon. 2010. Bidirectional sex change in marine fishes. In: K. S. Cole (Ed.) Reproduction and sexuality in marine fishes: patterns and processes. Berkeley, California: University of California Press. pp: 241-271.

Nakabo, T. (ed.) (2018). The Natural History of the Fishes of Japan. Tokyo: Shogakukan. (in Japanese)

Policansky, D. (1982). Sex change in plants and animals. *Annual Review of Ecology and Systematics*, *13*, 471-495. https://doi.org/10.1146/annurev.es.13.110182.002351

Quinitio, G. F., N. B. Caberoy, & D. Reyes. (1997). Induction of sex change in female *Epinephelus coioides* by social control. *Israeli Journal of Aquaculture*, *49*, 77-83.

Sato, T., M. Kobayashi, T. Kurihara, T. Takebe, N. Hirai, K. Okuzawa, S. Sawaguchi, T. Matsubara,

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Y. Akita, A. Ebisawa, I. Ohta, M. Uehara, T. Shimose, T. Yamaguchi, R. Shinoda, M. Koiso, & K. Teruya. (2018). Impact of fishing losses of males on the reproductive output of the large protogynous fish, *Choerodon schoenleinii*. *Marine Ecology Progress Series*, *602*, 225-235. https://doi.org/10.3354/meps12675

Warner, R. R. (1975). The adaptive significance of sequential hermaphroditism in animals. *The American Naturalist*, *109*, 61-82. https://doi.org/10.1086/282974

White, C. R., P. Cassey, & T. M. Blackburn. (2007). Allometric exponents do not support a universal metabolic allometry. *Ecology*, *88*, 315-323. https://doi.org/10.1890/05-1883

Yamaguchi, S. (2016). Time required for sex change in teleost fishes: hormonal dynamics shaped by selection. *Journal of Theoretical Biology*, *407*, 339-348. https://doi.org/10.1016/j.jtbi.2016.07.012

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

TABLES

Table 1. Effects of body mass, gonad type, and their input orders on the time required for sex change (T_S) in each sex-changing direction, as determined by the ordinary least squares multiple regression method.

First inputted variable	Direction	Source	*df*	*MS*	*F*	*P*
Body mass	female to male	**Body mass**	**1**	**0.36**	**6.25**	**0.03**
		Gonad Type	1	0.05	0.90	0.37
		Residuals	9	0.06		
	male to female	**Body mass**	**1**	**0.45**	**6.55**	**0.03**
		Gonad Type	1	0.14	2.01	0.18
		Residuals	11	0.07		
Gonad type	female to male	**Gonad Type**	**1**	**0.40**	**7.04**	**0.03**
		Body mass	1	0.01	0.11	0.75
		Residuals	9	0.06		
	male to female	**Gonad type**	**1**	**0.57**	**8.36**	**0.01**
		Body mass	1	0.01	0.20	0.66
		Residuals	11	0.07		

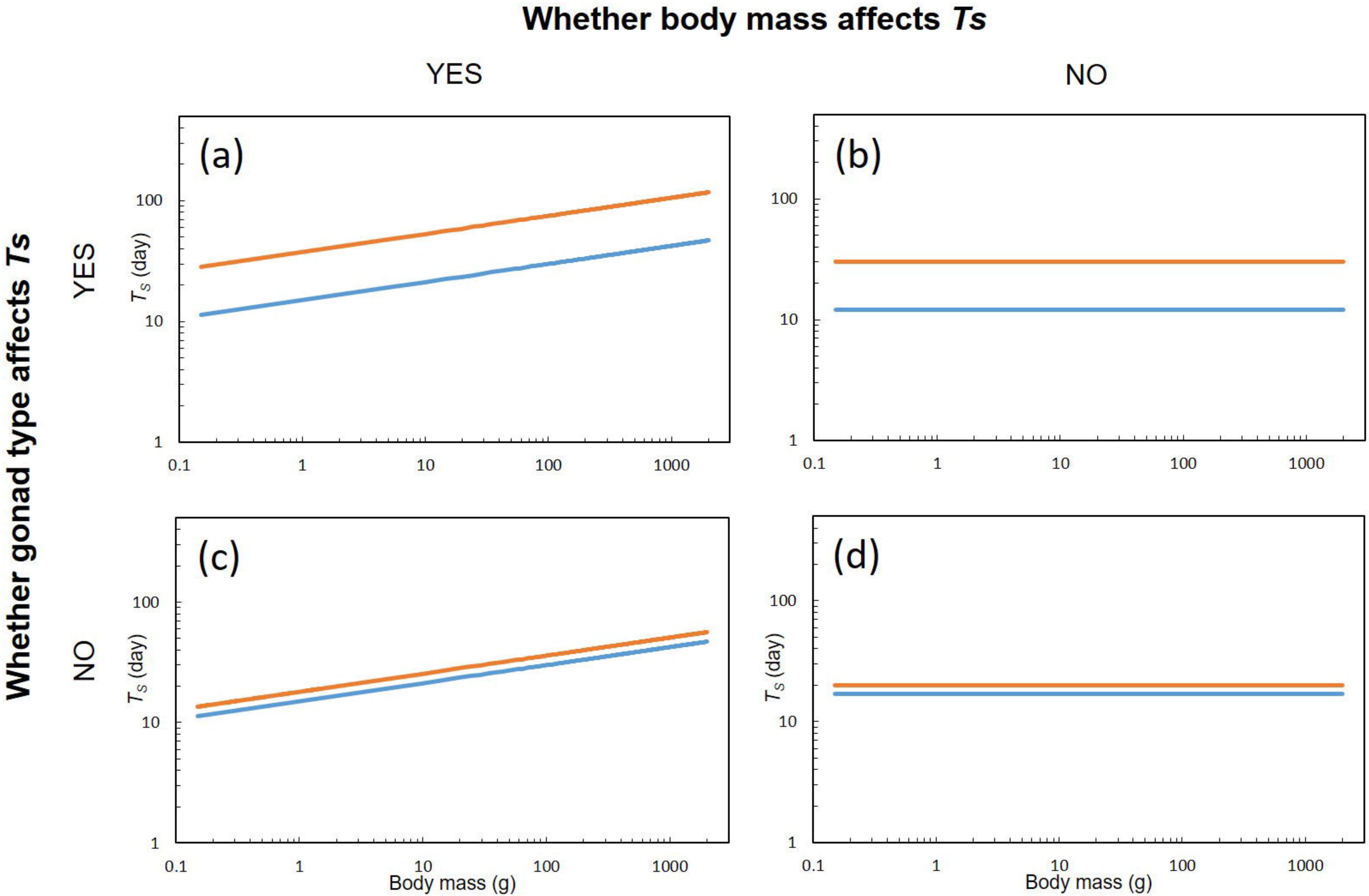
Significant differences ($p < 0.05$) are in bold; *df*, degrees of freedom; *MS*, mean square. T_S and body mass were $\log_{10}$-transformed.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.29.458130; this version posted August 31, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

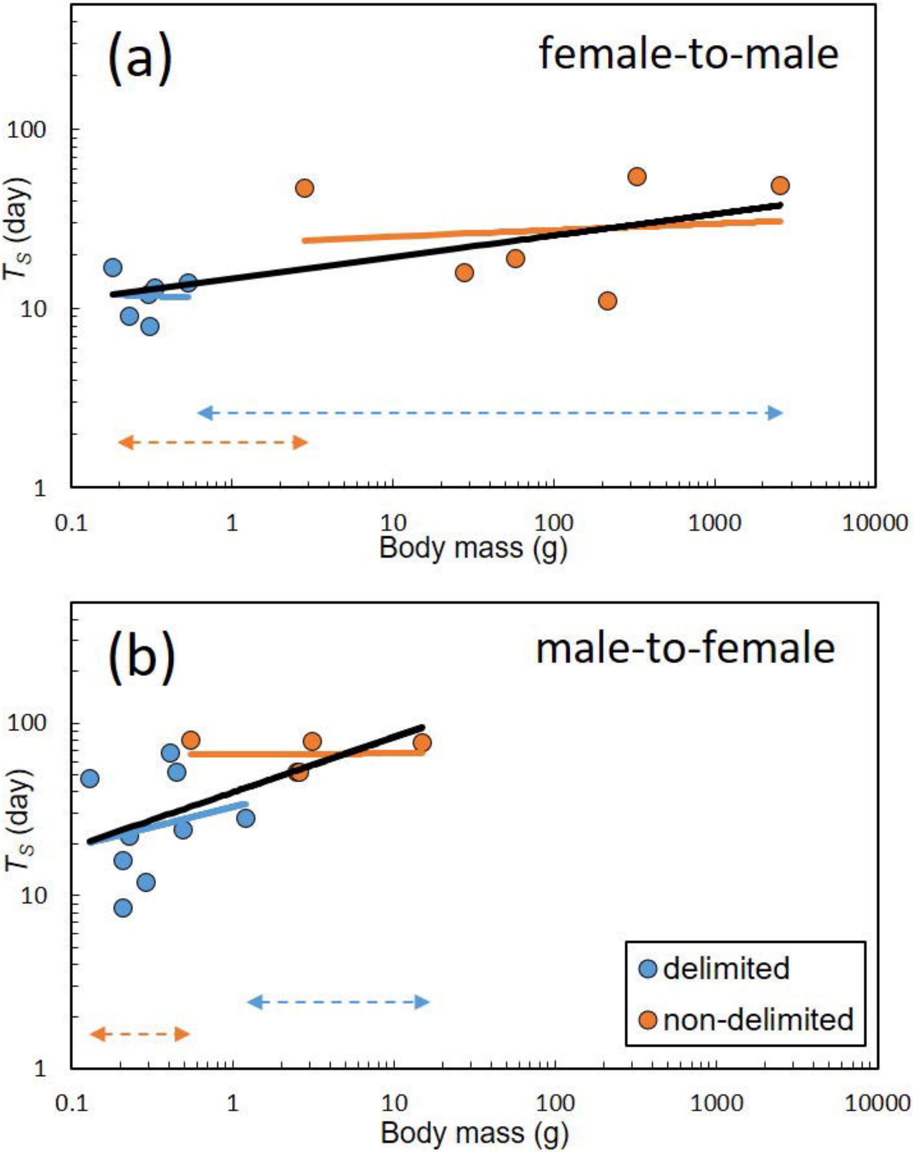
FIGURE CAPTIONS

Figure1. Four possible scenarios of the effects of gonad type and body mass on the time required for sex change (T_S). Note that only the scenarios for one sex changing direction are shown, because the scenarios are basically same for both directions. The blue and orange lines represent the expected regression lines for delimited and non-delimited species, respectively. (a) The scenario where both gonad type and body mass determine T_S. (b) The scenario where gonad type alone determines T_S. (c) The scenario where body mass alone determines T_S. (d) The scenario where neither gonad type nor body mass affects T_S.

Figure 2. Relationship between body mass and time required for sex change (T_S) in (A) female-to-male direction and (B) male-to-female direction. Data are plotted by species. The black solid lines are regression lines for female-to-male ($T_s = 14.7 \times mass^{0.12}$) and male-to-female sex changes ($T_s = 39.8 \times mass^{0.32}$). The blue and orange solid lines are regression lines separately calculated for delimited and non-delimited species, respectively. The values of these four slopes are not significantly different from zero ($p > 0.05$). The blue and orange double-headed dotted arrows represent body mass ranges lacking in our dataset for delimited and non-delimited species, respectively.



Whether body mass affects Ts
YES
NO
Whether gonad type affects Ts
YES
NO
(a)
(b)
(c)
(d)
T_S (day)
1
10
100
0.1
1
10
100
1000
Body mass (g)



(a)
female-to-male
100
10
1
T_S (day)
0.1
1
10
100
1000
10000
Body mass (g)
(b)
male-to-female
100
10
1
T_S (day)
0.1
1
10
100
1000
10000
Body mass (g)
delimited
non-delimited