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RESEARCH ARTICLE

A comparison of two methods for estimating critical swimming speed (U_{crit}) in larval fathead minnows: the laminar flow assay and the spinning task assay

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ABSTRACT

Critical swimming speed (U_{crit}) is considered a good predictor of swimming capabilities in fish. To estimate U_{crit}, a fish is exposed to an incrementally increasing laminar flow of water until it cannot maintain its position against the current. The spinning task assay has been proposed as an alternative method to traditional laminar flow methods; however, these methods have not been directly compared. Thus, the goal of this study was to determine whether the spinning task assay is a suitable alternative to traditional laminar flow assays. To that end, the performance of fathead minnows in each assay was compared at three time points (14, 19 and 24 days post-fertilization, dpf). In 14 dpf fish, U_{crit} estimates were similar regardless of the assay used. However, at 19 and 24 dpf, U_{crit} estimates derived from the two assay types were significantly different. This indicates that the assays are not equivalent to one another and that the spinning task assay is not a suitable alternative to the laminar flow assay for the determination of U_{crit}.

KEY WORDS: Swimming ability, Early life stage, Fish larvae, Cardiovascular performance

INTRODUCTION

Critical swimming speed (U_{crit}; Brett, 1964) has been directly tied to cardiac output of fish and is considered to be a good predictor of swimming capability (Claireaux et al., 2005). As such, it has been utilized across disciplines to demonstrate the impacts of genetics (Wakamatsu et al., 2019), morphology (Wakamatsu et al., 2019), growth rate (Kolok and Oris, 1995), environmental conditions (Penghan et al., 2014), exercise training (Shrivastava et al., 2018), social conditions (Wiwchar et al., 2018) and contaminant exposure (Mager et al., 2014) on swimming ability and/or cardiovascular fitness in a number of species. The most common method of estimating U_{crit} is through the use of a swim flume in which the fish is confined to a portion of the flume and subjected to a constant laminar current (Brett, 1964). The speed of the water flow is incrementally increased until the fish becomes fatigued and can no longer maintain its position against the current. The speed of the water flow and the amount of time required to push the fish to fatigue are then used for the calculation of U_{crit}:

$$U_{crit} = U + \left[U_i\left(\frac{T}{T_i}\right)\right], \quad (1)$$

where U is the highest water velocity at which the fish is able to maintain its position against the current for a full interval (cm s^{-1}), U_i is the speed by which the water speed is increased at each interval (cm s^{-1}), T is the time that the fish spent at its highest velocity (U) before fatigue (s), and T_i is the prescribed interval time between each incremental increase in velocity (s) (Brett, 1964). Methods for performing laminar flow assays are well documented and have been used to assess the cardiovascular performance in a wide variety of fish species, including adults of smaller species such as zebrafish (*Danio rerio*), killifish (*Fundulus heteroclitus*) and fathead minnows (*Pimephales promelas*); juveniles of larger species such as sockeye salmon (*Oncorhynchus nerka*), sturgeon (*Acipenser brevirostrum*) and mahi-mahi (*Coryphaena hippurus*); and larvae from marine reef and pelagic species such as clownfish (*Amphiprion melanopus*), red drum (*Sciaenops ocellatus*) and damselfish (*Neopomacentrus bankieri*) and freshwater species such as trout cod (*Maccullochella macquariensis*) and perch (*Macquaria ambigua*) (Bellwood and Fisher, 2001; Brett, 1964; Brown et al., 2017; Downie and Kieffer, 2017; Downie et al., 2020; Faria et al., 2009; Kopf et al., 2014; Leis and Fisher, 2006; Mager and Grosell, 2011; Mager et al., 2014; Silva et al., 2015; Stobutzki and Bellwood, 1994; Wiwchar et al., 2018).

A potential alternative to the laminar flow assay is the spinning task assay. The spinning task assay is a modification of an existing respirometry method (Munday et al., 2009; Nilsson et al., 2007) originally developed to assess the motor coordination and swimming behavior of zebrafish (Blazina et al., 2013). However, it has also been proposed as an alternative method for estimating U_{crit} (Usui et al., 2018). In this assay, a fish is placed in a 1 liter beaker with a magnetic stir bar. The beaker is then placed on a magnetic stir plate and the speed of the stir bar is incrementally increased, generating a current for the fish to swim against. As in the laminar flow assay, the current is increased until the fish is fatigued and can no longer maintain its position against the current, and the speed at which the fish is fatigued, as well as how long the fish maintained its position at that speed, are then used for the calculation of U_{crit}. The spinning task assay has been proposed as a more feasible alternative to the laminar flow assay, overcoming some potential obstacles such as the cost and relatively large footprint of commercial recirculating flumes, the challenges associated with the construction of 'home-made' flumes, and the time required to assess multiple fish (Killen et al., 2017).

While laminar flow and spinning task assays have been compared for the purposes of determining metabolic rate (Rummer et al., 2016), to our knowledge there have been no direct comparisons between these assays when utilized for the purpose of estimating U_{crit}. In the absence of such comparisons, it remains unclear whether the spinning task assay can be used in place of the traditional laminar flow assay for the estimation of U_{crit}. Therefore, the goal of this study was to determine whether the spinning task

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assay is a suitable alternative to the traditional laminar flow assay for calculating U_{crit} in larval fish. To achieve this goal, larval–juvenile fathead minnows were subjected to both assays so that U_{crit} values could be calculated and compared. Because previous studies have shown that swim performance is influenced by body size, U_{crit} was measured in 14, 19 and 24 days post-fertilization (dpf) fish to ensure that potential differences in assay performance due to differences in body size were accounted for.

MATERIALS AND METHODS

General study design

All procedures involving fathead minnows, *Pimephales promelas* (Rafinesque 1820), were conducted in accordance with Texas Christian University (TCU) IACUC-approved methods (protocol 18-12). Newly hatched (<24 h) eleutheroembryos were transferred to three 1 liter beakers containing 650 ml of clean water for a total of 25 fish per beaker. Beakers were covered with clear acrylic to reduce evaporation and maintained at ∼27°C in a Panasonic MIR-254 incubator with gentle aeration. Water changes of 80% were done daily and fish were fed newly hatched live *Artemia* nauplii twice daily. On each assessment day (14, 19 and 24 dpf), one beaker of fish was selected and 20 of the fish were randomly divided into two groups of 10; one group was designated for the laminar flow assay and one for the spinning task assay (Fig. 1). One hour after the morning feeding, 5 fish from the laminar flow group were subjected to the laminar flow assay, while 5 fish from the spinning task group were subjected to the spinning task assay. This was repeated until all 10 fish from each group had been assessed. After the groups had completed their respective assays, the fish were allowed to rest for 3 h. After this rest period, fish were fed again to account for the potential influence of feeding on swim performance and an hour later the group that had previously completed the laminar swim assay was subjected to the spinning task assay and vice versa. Individuals were tracked to allow for direct comparison of the two assay types. After the fish had completed both assays, wet mass and total length were measured, as size is known to impact U_{crit}. Mass and length data for fish utilized at each time point are presented in Table 1. Different groups of fish were used on each assessment day to limit the potential impacts of exercise training (Palstra et al., 2010; Usui et al., 2018). In cases where U_{crit} could not be calculated due to a lack of failure (i.e. the swimming capabilities of the fish exceeding the maximum water velocity within a given assay), the maximum possible U_{crit} was calculated for that fish.

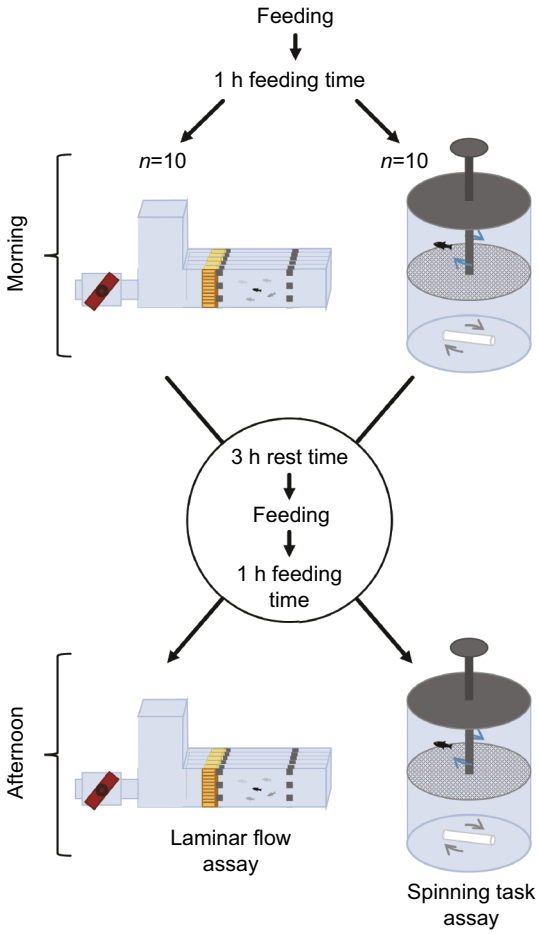

Fig. 1. Experimental design. Schematic diagram showing the general experimental design used to compare the laminar flow and spinning task assays for the estimation of critical swimming performance in larval–juvenile fathead minnows.

Laminar flow assay

The swim flume used for the laminar flow assay was designed and built based on descriptions by Stobutzki and Bellwood (1994, 1997) and Faria et al. (2009). Briefly, the six-lane flume was made from clear acrylic according to the specifications shown in Fig. S1. Each parallel lane (18 cm×3 cm×11 cm length×width×depth) was enclosed by mesh at either end to prevent fish from leaving their lane, and flow straighteners (a bundle of 4 cm long plastic straws) were positioned directly upstream of each lane to reduce turbulence and promote laminar flow. To maintain constant flow, water was pumped through the flume via a recirculating system powered by a 2600 gph (∼164 l min^{-1}) pump at 14 dpf and a 4400 gph (∼278 l min^{-1}) pump at 19 and 24 dpf. A larger pump was utilized at 19 and 24 dpf because of the increased swimming capabilities of the fish as they developed. Flow rate was regulated by a ball valve, which was calibrated based upon the position of the valve handle relative to a protractor mounted behind it. Speed was calibrated by determining how long it took for water flowing from the valve through the flume to fill a 5 liter container divided by the total cross-sectional area of the flume and the number of lanes (Faria et al., 2009). At each setting, the flow was measured 3 times and the resultant average flow rate was used to generate a calibration curve demonstrating the relationship between the angle of the valve handle and water velocity. The flow speeds used in the experiment varied from 2.5 to 24.5 cm s^{-1} at 14 dpf and from 3 to 63 cm s^{-1} at 19 and 24 dpf. The water current in the flume was increased at 2 min intervals (T_i) by 2 cm s^{-1} (U_i) on day 14 (corresponding to 2.0 BL s^{-1}, where BL is body length) and by 6.5 cm s^{-1} (U_i) on days 19 and 24 (corresponding to 5.6 and 4.5 BL s^{-1}, respectively). Fish were given a 5 min acclimation period at the lowest speed to allow them to adjust to the flume prior to increasing flow rate (Stobutzki and Bellwood, 1994; Faria et al., 2009). Failure was deemed to occur when a fish was unable to pull itself away from the mesh at the end of a lane, even after gentle prodding with a plastic transfer pipette.

Spinning task assay

The spinning task assay was carried out using the methods described by Usui et al. (2018). Briefly, 750 ml of water was added to a 1-liter French press coffee maker (IKEA) with the mesh guard submerged so that ∼400 ml of water was displaced above the mesh. A magnetic stir bar was added to the bottom compartment of each coffee maker prior to depression of the mesh guard and

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Table 1. Mass, length, and absolute and relative critical swimming speed (U_{crit}) for fish at 14, 19 and 21 days post-fertilization (dpf)

			Spinning task assay			Laminar flow assay		
Age (dpf)	Mass (mg)	Length (mm)	% Fatigued	Absolute U_{crit} (cm s^{-1})	Relative U_{crit} (BL s^{-1})	% Fatigued	Absolute U_{crit} (cm s^{-1})	Relative U_{crit} (BL s^{-1})
14	5.8±1.2^{C}	10.1±0.1^{C}	90	15.4±0.8^{A,B}	15.4±0.9^{A}	95	15.6±0.8^{C}	15.5±0.8^{B}
19	11.0±0.4^{B}	11.3±0.1^{B}	95	12.4±0.8^{B,*}	11.0±0.7^{B,*}	60	50.1±3.4^{B}	44.5±3.1^{A}
21	26.8±1.7^{A}	14.4±0.3^{A}	70	17.2±1.2^{A,*}	12.1±0.9^{B,*}	15	64.1±0.6^{A}	44.8±0.8^{A}

All metrics are presented as means±s.e.m. The percentage of fish that were successfully fatigued is also presented. n=20 for each age class. Different superscript letters indicate significant differences across ages within each metric of swim performance. *Significant differences between U_{crit} values derived from the spinning task assay relative to the laminar flow assay within each age group.

each French press was placed on a magnetic stir plate that had been calibrated for speed to ensure uniformity across multiple apparatuses. Because room temperature was ∼19°C, the water temperature in each French press was checked prior to the initiation of all trials and water was replaced between each trial to maintain temperatures of ∼26°C. A total of five individual French press swim chambers and stir plates were used in the present study. The water velocity at each speed setting on the stir plate were determined by monitoring a free-floating 1×1 cm piece of tape on the surface of the water and counting the number of rotations during a 30 s interval. To allow for direct comparisons between the results of the spinning task and laminar flow assays, the median circumference of each French press was estimated and used to convert water velocity from rpm to cm s^{-1}. Water velocity varied from 1.6 to 22.6 cm s^{-1}. Fish were allowed 5 min at 1.6 cm s^{-1} to acclimate to the swim chamber. After the initial acclimation period, the water speed was increased by 2 cm s^{-1} (corresponding to 2.0, 1.8 and 1.4 BL s^{-1} at 14, 19 and 21 dpf, respectively) every 2 min until failure. Failure was defined as the time at which the fish was unable to maintain its position against the water current for ≥30 s.

Statistical analysis

Critical swimming speed U_{crit} is reported as both body lengths per second (BL s^{-1}) and absolute values (cm s^{-1}), as both values are frequently used in the literature. Regression analysis was utilized to evaluate the influence of fish length on U_{crit} as estimated from the laminar flow and spinning task assays. The slopes of the resultant regression lines were then compared via analysis of covariance (ANCOVA) with assay type as a factor and length as a covariate to assess the relative performance of the two assays. In addition, direct comparisons of laminar flow U_{crit} values and spinning task U_{crit} values within an age group were made using a matched-pairs t-test. All statistical analysis was done using the statistical software package JMP 14.0.

RESULTS

Table 1 shows the size and swim performance metrics of fathead minnow larvae measured at 14, 19 and 21 dpf. Significant differences in mass and length at each age were noted (Wilcoxon test, P<0.01 for both metrics). Specifically, 21 dpf fish were significantly larger than 14 and 19 dpf fish and 19 dpf fish were significantly larger than 14 dpf fish. Significant differences in absolute U_{crit} as a function of age were noted for both the spinning task (ANOVA, P<0.01) and laminar flow (Wilcoxon test, P<0.01) assays. For the spinning task assay, the absolute U_{crit} values for 14 and 19 dpf larvae were similar to one another, as were those of 14 and 21 dpf larvae. However, the absolute U_{crit} values of 21 dpf larvae were significantly greater than those of 19 dpf larvae. In contrast, the absolute U_{crit} values determined for 12, 19 and 21 dpf larvae via the laminar flow assay were all significantly different from one another, with absolute U_{crit} increasing with age. Significant differences in relative U_{crit} as a function of age were also noted (spinning task assay, ANOVA, P<0.01; laminar flow assay, Wilcoxon test, P<0.01). There were no differences in the relative U_{crit} of 14 and 19 dpf larvae as measured in the spinning task assay; however, both were significantly lower than that of 14 dpf larvae. When relative U_{crit} was determined via the laminar flow assay, there were no differences in the U_{crit} values obtained for 19 and 21 dpf larvae, but both were significantly higher than that of the 14 dpf larvae.

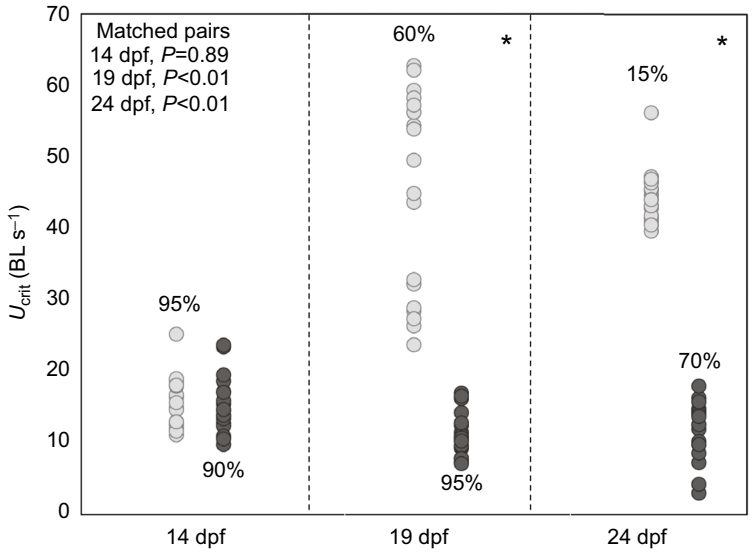

Fig. 2. Relative critical swimming speed (U_{crit}) estimated via the laminar flow and spinning task assays. Light circles represent U_{crit} values derived from the laminar flow assay and dark circles represent U_{crit} values derived from the spinning task assay at 14, 19 and 24 days post-fertilization (dpf; n=20 for each age class). The percentage of fish that reached failure is indicated, which is required for determining U_{crit}. *Significant increase in laminar flow assay U_{crit} values at that time point when compared with 14 dpf. BL, body lengths.

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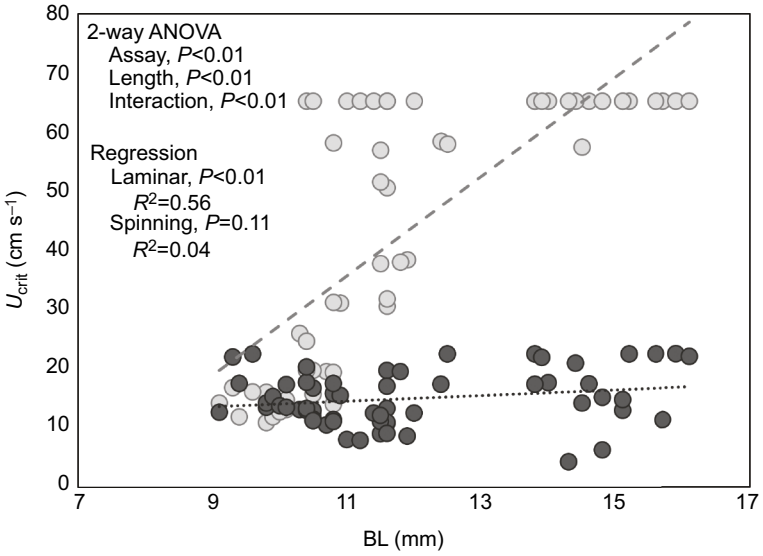

Fig. 3. Absolute U_{crit} estimated from the laminar flow and spinning task assays as a function of body length. Light circles represent U_{crit} values derived from the laminar flow assay, dark circles represent U_{crit} values derived from the spinning task assay (n=60). Dashed line represents trend line for U_{crit} estimates derived from the laminar flow assay, dotted line represents trend line for U_{crit} values derived from the spinning task assay.

Direct comparisons between spinning task assay and laminar flow assay U_{crit} estimates revealed a lack of significant differences at 14 dpf (Table 1, Fig. 2; matched-pairs t-test, P=0.89). However, significant differences between the U_{crit} values measured by the two assays were detected at 19 and 24 dpf (Fig. 2; matched-pairs t-test, P<0.01 in both cases). At both 19 and 21 dpf, U_{crit} values obtained via the laminar flow assay were significantly higher than those obtained via the spinning task assay. The noted differences in U_{crit} values measured by the two assays were further explored via ANCOVA analysis, which revealed that the assay type, body length and interaction between the two had a significant effect on U_{crit} (Fig. 3; ANCOVA, P<0.01 for all). Specifically, there was a significant positive correlation between total length and U_{crit} as estimated via the laminar flow assay (Fig. 3; P<0.01, R^2=0.56), but not between length and U_{crit} as measured by the spinning task assay (Fig. 3; P=0.11). Further, linear regression analysis of laminar flow U_{crit} and spinning task U_{crit} revealed a statistically significant, yet weak, negative correlation between the U_{crit} values (Fig. 4; P<0.01, R^2=0.22).

DISCUSSION

Biological comparison

In general, there are few data available regarding the performance of larval freshwater fish in the laminar flow assay. To our knowledge, U_{crit} estimates for larval fathead minnows have not been reported elsewhere; however, several studies have measured U_{crit} in juvenile (~55–65 dpf) fathead minnows. A comparison of larval and juvenile fathead minnow U_{crit} values, as determined via the laminar flow assay, showed that the U_{crit} values of 19 and 24 dpf larvae in the present study were higher than those reported for juveniles (6.18±0.8 BL s^{-1}: Goertzen et al., 2011; 27.6±0.8 BL s^{-1}: Mager and Grosell, 2011; 27.5–40.8 cm s^{-1}: Kolok et al., 2004). The lack of consistency in the values obtained for juveniles, combined with the fact that higher U_{crit} estimates were obtained for larvae than for juveniles despite the known relationship between size and U_{crit}, suggests that the noted differences in U_{crit} stem from differences in the specific methods utilized across studies (Downie and Kieffer, 2017; Kern et al., 2018). For example, the studies by Kolok et al. (2004) and Mager and Grosell (2011) used habituation periods of 60–90 min and intervals of 20–30 min, whereas a habituation period of 5 min and interval of 2 min were used in the present study. As such, differences in laminar flow swim assay methods may preclude meaningful cross-study comparisons. A study by Kopf et al. (2014), which investigated the swim performance of six freshwater larval fish species using similar methods to those of the current study (e.g. habituation period of 5 min and interval of 5 min), allows for comparisons of larval

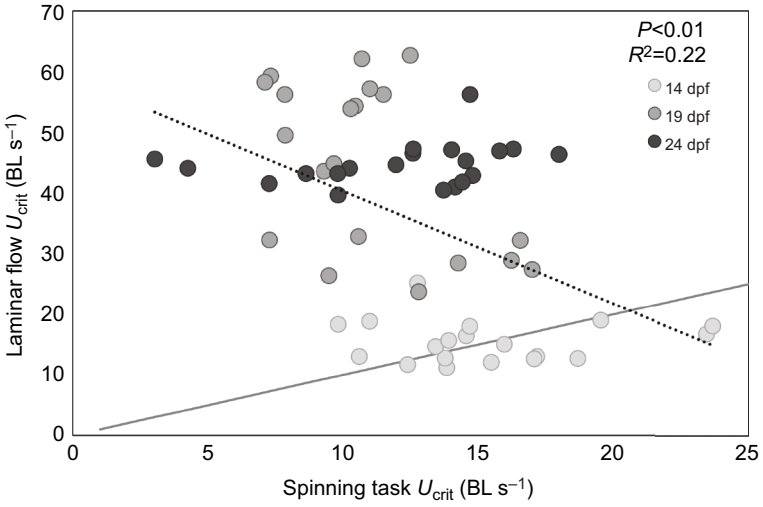

Fig. 4. Linear regression of relative U_{crit} estimated from the laminar flow and spinning task assays. Circle shading indicates 14, 19 and 24 dpf (n=60). Solid line represents a theoretical 1:1 relationship between variables, dotted line represents trendline for results.

fathead minnows and other larval species. Such comparisons reveal that fathead minnow larvae have U_{crit} values similar to those of other species with well-developed and highly mobile larvae (i.e. trout cod (*M. macquariensis*: 44.6 BL s^{-1}) and Murray cod (*Maccullochella peelii*: ~35 BL s^{-1}), but higher than those of species with less well-developed larvae such as perch (*M. ambigua* and *Bidyanus bidyanus*), carp gudgeons (*Hypseleotris* spp.), and Australian rainbowfish (*Melanotaenia fluviatilis*; Kopf et al., 2014).

Assay comparison

The lack of significant differences in the U_{crit} values measured at 14 dpf between the spinning task assay and laminar flow assay may suggest that the spinning task assay is comparable to the laminar flow assay. However, the significant differences noted at 19 and 24 dpf suggest otherwise, especially when the fact that the extent of the differences between U_{crit} values is likely an underestimate given that 40% and 85% of fish subjected to the laminar flow assay at 19 and 24 dpf, respectively, were capable of swimming at speeds beyond 63 cm s^{-1} (the highest water velocity possible in the laminar flow assay; Table 1). This is supported by the results of the ANCOVA analysis, which revealed significant differences in U_{crit} values obtained via the different assays. In addition, the ANCOVA analysis indicated a significant interaction between assay type and the body length of the fish, suggesting assay-specific differences in the relationship between body size and U_{crit}. Previous research has repeatedly demonstrated that the U_{crit} values of larval fish increase with age and size (Faria et al., 2009; Silva et al., 2015); thus, a significant positive correlation between body length and U_{crit} would be expected. Such a relationship was observed when U_{crit} values were estimated via the laminar flow assay, indicating the validity of the laminar flow assay for the evaluation of swim performance. However, no such correlation between length and U_{crit} values was observed when the spinning task assay was used to evaluate swim performance. Thus, it can be concluded that the spinning task assay is invalid for measuring swim performance in fish. Linear regression analysis directly comparing spinning task U_{crit} estimates with laminar flow U_{crit} estimates also supports this conclusion. If the performance of the assays were equivalent to one another, a statistically significant nearly 1:1 positive correlation would be expected. Here, a statistically significant and weak negative correlation was observed, providing additional evidence that the spinning task assay should not be considered an equivalent alternative to the laminar flow assay for U_{crit} determination.

Overall, the differences between U_{crit} estimates derived from the assays, the lack of a relationship between U_{crit} values generated by the spinning task assay and fish length, and the lack of a positive correlation between U_{crit} estimates generated by the two assay types indicates that the spinning task assay is not estimating U_{crit} in a manner consistent with that of the laminar flow assay. This is further supported by the results of Rummer et al. (2016), which showed differences in estimated maximal metabolic rate (MMR) when two respirometry methods (laminar current versus circular current) were compared. The authors hypothesized that lower MMR estimates generated via circular respirometry methods were due to fish experiencing muscular exhaustion (stemming from imbalanced muscle use due to constant rotational motion), rather than true fatigue. In addition, it is likely that the speed of the current in spinning task assay chambers is not as consistent as it is in the laminar flow chambers as a result of varying speeds across the area of the vortex (i.e. velocity at the outermost edge of the chamber may not be equivalent to that at the center of the chamber) (Blazina et al., 2013; Nilsson et al., 2007; Rummer et al., 2016). Overall, these results indicate that the spinning task assay is not a viable replacement for the laminar flow assay for the determination of U_{crit}, as estimates of U_{crit} generated via the spinning task assay did not exhibit a positive correlation with body size as would be anticipated. Furthermore, the potential for the spinning task assay to underestimate U_{crit} limits its utility. As such, estimates of U_{crit} are best made using traditional laminar flow assays.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.C.K., M.K.S.J.; Methodology: J.C.K.; Formal analysis: J.C.K.; Investigation: G.S.L., M.K.S.J.; Resources: M.K.S.J.; Data curation: M.K.S.J.; Writing - original draft: J.C.K., G.S.L.; Writing - review & editing: J.C.K., G.S.L., M.K.S.J.; Visualization: J.C.K.; Supervision: M.K.S.J.; Project administration: J.C.K., M.K.S.J.; Funding acquisition: J.C.K., G.S.L., M.K.S.J.

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