Forced sustained swimming exercise at optimal speed enhances growth of juvenile yellowtail kingfish (Seriola lalandi)

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INTRODUCTION

Aquaculture is facing an increasing demand for sustainably produced fish. There is a great need to optimize the conditions for fish growth, but without compromising health and welfare. Currently, significant numbers of commercially produced species of fish suffer from impaired well-being and high mortality (e.g., Castro et al., 2011, 2013). This may, at least partly, be explained by the fact that fishes cannot display their normal swimming behavior due to high densities or low water flow. A promising natural, non-invasive and economical tool to enhance growth may be the induction of swimming exercise at optimal swimming speeds (Palstra and Planas, 2011). Evidence suggests that the health and welfare of swimming fish is improved, not compromised (Castro et al., 2013; reviewed by Huntingford and Kadri, 2013).

Sustained swimming exercise improves growth in several teleostean fishes (Jobling et al., 1993; Davison, 1997; Palstra and Planas, 2011; Davison and Herbert, 2013). Exercise enhanced growth performance in salmonids, such as brook trout Salvelinus fontinalis (Leon, 1986; East and Magnan, 1987), brown trout Salmo trutta (Davison and Goldspink, 1977; Bugeon et al., 2003), rainbow trout Oncorhynchus mykiss (Greer Walker and Emerson, 1978; Houlihan and Laurent, 1987), Arctic charl Salvelinus alpinus (Christiansen et al., 1989, 1992; Grünbaum et al., 2008), and Atlantic salmon Salmo salar (Totland et al., 1987; Jørgensen and Jobling, 1993; Castro et al., 2011). Growth stimulation by swimming exercise has also been reported for non-salmonid species like yellowtail kingfish Seriola lalandi (Brown et al., 2011); gilthead seabream Sparus aurata (Ibarz et al., 2011; Sánchez-Gurmaches et al., 2013), whiting Merlangius merlangus (Hammer, 1994), striped bass Morone saxatilis (Young and Cech, 1993, 1994), qingbo Spinibarbus sinensis (Li et al., 2013) and the Amazonian species matrinx Brycon amazonicus (Arbeláez-Rojas and Moraes, 2010) and pacu Piaractus mesopotamicus (Da Silva Nunes et al., 2013); also, zebrafish Danio rerio grow better when forced to swim (Palstra et al., 2010). The skeletal muscle thereby undergoes morphometrical and biochemical changes in response to exercise (Johnston and Moon, 1980; Davison, 1997; Johnston, 1999; Bugeon et al., 2003; Martin and Johnston, 2005; Rasmussen et al., 2013). Exercise increases muscle transcriptional activity underlying these changes, in particular genes involved...
The first objective of this study is to assess the swimming performance of juvenile yellowtail kingfish, specifically the changes in optimal swimming speed during juvenile development. We subjected three size classes of juveniles to swim performance tests in swim-tunnels equipped with respirometers. Secondly, we then applied the relation between size and $U_{\text{opt}}$ to determine the effects of long-term forced and sustained swimming at $U_{\text{opt}}$ on growth performance. For this purpose we subjected two groups to either a water flow forcing fish to swim at $U_{\text{opt}}$, or to a low water flow that allows spontaneous activity. A newly designed 3600 L oval-shaped recirculating swim-flume was used to induce straight line swimming. The physiological consequences of exercise on growth were assessed by quantification of differences in size and blood flow. Importantly, both groups of fish were fed equal rations to avoid that feed intake would act as confounder in explaining anticipated physiological differences. In the light of earlier results (Brown et al., 2011), we hypothesize that forced sustained, straight line swimming exercise at $U_{\text{opt}}$ will enhance growth performance of yellowtail kingfish and increase blood flow.

MATERIALS AND METHODS

ETHICS

All experiments were performed in accordance with relevant guidelines and regulations. Protocols used complied with the current laws of the Netherlands and were approved by the Animal Experimental Committee (DEC) of the Wageningen UR in Lelystad (The Netherlands) under numbers 2012012 and 2013162.

SWIM PERFORMANCE TESTS AND RESPIROMETRY IN SWIM-TUNNELS

Experimental fish and conditions

Juvenile yellowtail kingfish ($n = 33$; $BL = 88 \text{ mm}$, $BW = \sim 8 \text{ g}$) were obtained from the farm Silt BV (IJmuiden, the Netherlands). They were transported from the farm to the IMARES facilities in Yerseke by truck within 3 h. Fish were housed under similar conditions as in the hatchery: in natural seawater that was mixed with tap-water to 25.1 ± 0.1‰, at 23.4 ± 0.1°C and under a light regime of 16L:8D before and during the experimental periods. Fish were hand-fed three times per day at 7% BW day$^{-1}$ with commercial feed (Skretting, Boxmeer, The Netherlands). Water quality was monitored daily for $O_2$, pH, $NH_4$, $NO_2$, and $NO_3$. Oxygen levels were $7.54 \pm 0.08 \text{ mg L}^{-1}$, pH was $8.07 \pm 0.07$, $NH_4$-N averaged $0.27 \pm 0.05 \text{ mg L}^{-1}$, $NO_2$-N averaged $1.5 \pm 0.2 \text{ mg L}^{-1}$ and $NO_3$-N averaged $8.9 \pm 0.9 \text{ mg L}^{-1}$ over the 2.5 months experimental period. During three periods in time, sub-samples of this batch of fish were used for swim performance tests (size groups 1–3; Table 1).

Swim performance tests and respirometry

For the swimming experiments, two 127 L Blazka-type swim tunnels were used (van den Thillart et al., 2004). In each swim tunnel, water from the housing tank was circulating. A bypass with a galvanic oxygen electrode in a 4-channel respirometry system (DAQ-PAC-G4; Loligo Systems Aps, Tjele, Denmark) allowed registration of oxygen consumption. During respirometry, water in the swim-tunnels was recirculated and total oxygen content
The experiment was terminated when either the fish fatigued or when the fish had swum at 1.00 m s\(^{-1}\). The experiment was conducted in a 3600 L oval-shaped Brett-type swim-flume (3.0 \(\times\) 2.0 \(\times\) 1.0 m; Figure 1; Brett, 1964) placed in a climatized room. The whole water volume of the recirculating system was pumped in 1 h over a filter system consisting of a Hydrotech drum filter (model HDF 501-1P, Hydrotech AB, Vellinge, Sweden), a trickle filter (Fleuren & Nooijen BV, Nederweert, the Netherlands), a 200 L biological moving bed biofilm reactor (MBBR) and a protein skimmer (Sander Aquarientechnik, Uetze-Eltze, Germany), all connected to a 400 L sump. Additionally, water from the 400 L sump was continuously pumped over a UV-filter (Proclear UV30 Advantage, Tropical Marine Centre Ltd. Hertfordshire, UK) and a heat exchanger (Maxicool XGL18HDA, Maxicool BV, Wessem, the Netherlands, modified by Climate4.uz.nl, Valkenswaard, the Netherlands) to maintain water quality and water temperature, respectively. In one of the straight ends of the flume, two mesh fences (green polyester coated steel, 11 mm mesh size) were used to construct a compartment of 200 \(\times\) 70 cm (Figure 1C). This compartment was divided by a PVC sheet (10 mm thick), thereby creating two 525 L sub-compartments, each measuring 200 cm \((x') \times\) 35 cm \((y') \times\) 70 cm \((z')\) (length \(\times\) width \(\times\) depth). The resulting inner sub-compartment, where water flow was nil, was used to house the resting fish. The outer compartment, where water flow was maximal, was used to house the swimming fish. Ample water circulation to maintain water quality in the inner compartment was ensured by installing a pump (Aquaria Ocean Runner OR 6500, Aqua Medic, Loveland, CO, USA) in the curve that flushed water through the compartment at a rate of 6500 l h\(^{-1}\).

Water flow was generated at the start of the straight end opposite the two compartments by an impeller connected to an electric motor (KLEEdrive MS2 132M-4 B3 (7.5 kW), Bred. Klee A/S, Albertslund, Denmark; Figure 1). The motor was powered by an industrial inverter (IP66, model no. BFI-E2-34-0180-3F4#, Beijer Electronics, Malmö, Sweden) with an adjustable alternating current (AC) output frequency between 0 and 50 Hz. This set-up to generate water flow was designed and first tested by Loligo Systems ApS (Tjelle, Denmark). Additionally, a Speck pump (Badu 90/13, 0.55 kW with a capacity of 13 m\(^3\) h\(^{-1}\); Speck Pumps, Jacksonville, USA) continuously generated water circulation throughout the flume to safeguard water quality when the impeller was stationary.

Prior to the start of the experiment, water velocity in the swim compartment was measured using a downward-faced Vectrino acoustic Doppler velocimeter (ADV; Nortek AS, Rud, Norway) with its focal point at the center of the water column, 25 cm from the upstream fence. When the impeller was stationary, flow in the swimming compartment was measured. Inverter frequencies were subsequently increased with 0.5 Hz increments from 2.5 to 8.5 Hz, and after each increment, water flow was left to stabilize for 5 min, after which water velocities were measured in three dimensions (velocities \(u\), \(v\), and \(w\) in directions \(x\), \(y\), and \(z\), respectively, as described above) for 10 s with a sampling rate of 10 Hz.

To quantify turbulence throughout the compartment, the inverter was set to 7.0 Hz, corresponding to a mean water velocity in the swimming compartment of 79 cm s\(^{-1}\), after which water velocities were measured within the swimming compartment at three horizontal transects (25, 100, and 175 cm), three vertical transects (8, 17.5, and 27 cm) at three depths (15, 37, and 55 cm)
FIGURE 1 | The constructed swim-flume. (A) side-view; and (B) schematic overview. An industrial inverter provided an alternating current with adjustable frequency (Hz) to an electric motor, which in turn powered the impeller. Water was led through the swimming compartment, forcing fish \( n = 23 \) to swim at the optimal swimming speed. Fish \( n = 23 \) in the rest-compartment were not subjected to water flow and displayed spontaneous activity. To maintain high water quality in the rest-compartment, low water flow was generated by a small pump, which was placed in the curve outside the compartment. See text for detailed description.

which resulted in a total of 27 measurements. Using velocities \( u, v, \) and \( w \), the dimensionless turbulence intensity (TI) was calculated according to Liao and Cotel (2013) at each of the 27 locations:

\[
TI = \frac{u'}{\left( \overline{u}^2 + \overline{v}^2 + \overline{w}^2 \right)^{1/2}}
\]

where \( u' \) is the standard deviation of velocity \( u \) and \( \overline{u}, \overline{v} \) and \( \overline{w} \) are the average velocities in direction \( x, y \) and \( z \), respectively.

Experimental fish and conditions
Juvenile yellowtail kingfish \( n = 56 \) from the farm Silt BV (IJmuiden, the Netherlands) were randomly assigned to the rest-compartment (“REST”; \( n = 28 \)) or the swimming compartment (“SWIM”; \( n = 28 \)) of the flume and were acclimatized for 2 days. After acclimatization, fish were not fed for 24 h and then ten fish (five from each compartment) were randomly selected and sampled (as “START” group). Individual fish were anesthetized in clove oil and total length (TL in mm) and body weight (BW in g) were measured from which Fulton’s condition factor \( K \) was calculated, after which fish were euthanized by decapitation. The heart was dissected and heart weight (HW) was determined.

One day after the START group was sampled, the swimming trial commenced and the swimmers \( n = 23 \) were forced to swim at \( U_{\text{opt}} \) in a sustained manner while resters \( n = 23 \) were allowed to perform spontaneous activity at low water flow. Fish were fed commercial feed (Efico Sigma 570 No 6.5, Biomar A/S, Brande, Denmark), three times per day on weekdays and twice per day during weekends. The water flow was stopped during feeding sessions. First, the resters were fed until apparent satiation and the amount of feed given was determined. Then, swimmers were pair-fed and received the same amount as the resters. After feeding, fish were left for 15 min and then the water velocity was gradually increased to \( U_{\text{opt}} \). Fish were fed 2.65 ± 0.13 % BW d\(^{-1}\) and food conversion ratio (FCR) for both treatments was calculated as the ratio of biomass gain (wet weight in g) to total food intake (g).

Fish were checked for swimming behavior at least twice per day. Experimental fish did not show any visible signs of stress or fatigue, nor did infections, disease, or mortality occur. During the experiment, salinity was maintained at 26.2 ± 0.2 ‰ by mixing natural seawater (from the estuary Oosterschelde) with tap water. Mean water temperature was maintained at 23.6 ± 0.1°C. Oxygen gas was supplied through a ceramic diffuser downstream of the two compartments. Hundred percentage system volume was replaced daily to ensure high water quality. Water quality was monitored daily for \( O_2 \), \( pH \), \( NH_4 \) and \( NO_2 \), while \( NO_3 \) was checked seven times throughout the 18-day experimental period. Oxygen levels were 7.67 ± 0.07 mg L\(^{-1}\), \( pH \) was 7.42 ± 0.029, \( NH_4 \)-N averaged 0.8 ± 0.2 mg L\(^{-1}\) (min 0 mg L\(^{-1}\) and max 2.3 mg L\(^{-1}\)), \( NO_2 \)-N averaged 1.4 ± 0.2 mg L\(^{-1}\) (min 0.2 mg L\(^{-1}\) and max 3.1 mg L\(^{-1}\)) and \( NO_3 \)-N averaged 14.8 ± 3.5 mg L\(^{-1}\).
Gain, PW 52%; Frequency, B 18 MHz, Frequency, CFM 8 MHz, and where ventral artery, just downstream of the motionless against the ventral side of the fish near the head, in a solution gel (Parker Laboratories Inc., Fairfield, NJ, USA) and held on the table with the right lateral side facing upwards. The ultrasound flow was determined using the “El-Flow” function of the ADV. Blood flow was visualized using the brightness (B) mode, Colour Flow Mapping (CFM) and Pulse Wave (PW) with the following settings: Velocity 89%; Angle (θ) + 60°; Depth 6 cm, Gain, B 76%, Gain, CFM 70%, Gain, PW 52%; Frequency, B 18 MHz, Frequency, CFM 8 MHz, and Frequency, PW 8 MHz. Anesthetized fish were positioned on a table with the right lateral side facing upwards. The ultrasound transducer was covered with Aquasonic ultrasound transmission gel (Parker Laboratories Inc., Fairfield, NJ, USA) and held motionless against the ventral side of the fish near the head, in a longitudinal direction, allowing visualization of blood flow in the ventral artery, just downstream of the bulbus arteriosus (Figure S1). The scanning procedure was completed within 2 min. Blood flow was determined using the “El-Flow” function of the ADV. Using this function, the user manually traces the contour of the blood velocity graph and the width of the blood vessel is indicated. The ADV subsequently calculates the time average velocity as well as the cross-sectional area of the vessel, assuming a circular shape. Using these two parameters, the ADV calculates blood flow in mL min⁻¹. After ultrasonography, fish were measured for TL and BW, after which fish were euthanized by decapitation.

Size measurements were used to calculate weight-specific growth rates for both swimmers and resters:

\[
SGR_w = \left( \ln \left( W_f \right) - \ln \left( W_i \right) \right) \times \frac{100}{t}
\]

where \( W_f \) is the final average weight of either resters or swimmers (g), \( W_i \) is the initial average weight of the START group (g) and \( t \) is time between measurements (d).

RESULTS

Swimming Performance Tests and Respirometry in Swim-Tunnels

Size

Size parameters (BW, TL, FL, and K) significantly increased from one experimental group to the next \( (P < 0.05; \text{Table 1}) \).

Swimming behavior

Seven fish of the smallest size group 1 at speeds of 0.80 m s⁻¹ benefited from reduced drag by continuously swimming close to the walls of the swimming tunnels. One fish of group 1, two of group 2 and one of group 3 could not swim and appeared stressed in the swim-tunnels. Three fish of group 1 (critical swimming speed \( \text{U}_{\text{crit}} = 0.30 \pm 0.06 \text{ m s}^{-1} \) or \( 2.16 \pm 0.47 \text{ BL s}^{-1} \); calculated like Brett, 1964), three fish of group 2 (\( \text{U}_{\text{crit}} = 0.71 \pm 0.15 \text{ m s}^{-1} \) or \( 2.61 \pm 0.53 \text{ BL s}^{-1} \)) and one fish of group 3 (\( \text{U}_{\text{crit}} = 0.60 \text{ m s}^{-1} \) or \( 1.88 \text{ BL s}^{-1} \)) fatigued before swimming 1 h at 1 m s⁻¹. One fish of group 1 was able to swim 1 h at 1 m s⁻¹ (equal to 6.21 BL s⁻¹) without swimming close to the walls, as well as seven of group 2 (represents 58% of the fish; equal to 4.12 ± 0.15 BL s⁻¹) and seven of group 3 (represents 78% of the fish; equal to 3.21 ± 0.06 BL s⁻¹).

Cost of transport (COT) and optimal swimming speeds (\( \text{U}_{\text{opt}} \))

The calculated cost of transport (COT) values were plotted against the swimming velocities as polynomial \( \text{U} \)-shaped curves for each of the subsequent groups (Figures 2A–C). Only in case of the smallest size group 1 the polynomial curve was actually the best fitting \( (R^2 = 0.9935) \). For groups 2 and 3, most of the fish were still swimming at 1 m s⁻¹ so that the minimal COT value determining \( \text{U}_{\text{opt}} \) may not have been reached. Polynomial curves (for groups 2 and 3, respectively, \( R^2 \)-values of 0.9624 and 0.9669) were plotted to calculate \( \text{U}_{\text{opt}} \)-values for both groups. The average COT was higher in group 1 as compared to the fish of groups 2 and 3 (Figure 2). The polynomial for group 1 followed the equation \( y = 9.9503x^2 - 13.924x + 5.9924 \); for group 2 the equation was \( y = 1.9141x^2 - 3.1348x + 1.5051 \); and for group 3 the equation was \( y = 2.6024x^2 - 4.3019x + 2.044 \) (Table 2). The absolute \( \text{U}_{\text{opt}} \) (in m s⁻¹) increased for each of the subsequent size groups from 0.70 m s⁻¹ for group 1; to 0.82 m s⁻¹ for group 2; to 0.85 m s⁻¹ for group 3 (Table 2). The relative \( \text{U}_{\text{opt}} \) (in BL s⁻¹) decreased: 4.83 BL s⁻¹ for group 1, 3.25 BL s⁻¹ for group 2, and 2.73 BL s⁻¹ for group 3 (Table 2).

Swim Training Test in a Swim-Flume

Flow and turbulence

Once the impeller was stationary, flow in the swimming compartment was 8.06 ± 0.27 cm s⁻¹ as a result of the circulation generated by the Speck pump. Horizontal water velocity \( (u) \) in the swimming compartment increased proportionally to the AC-frequency of the inverter \( (R^2 = 0.9977; \text{Figure S2}) \). Turbulence intensity (TI) in the swim compartment was subsequently calculated as the average over the 27 measurements. At a mean water
velocity of 79 cm s$^{-1}$ in the swimming compartment, TI was 0.083 ± 0.0032.

**Swimming behavior**
Swimmers displayed rheotactic behavior, grouping together in a school near the upstream fence of the compartment. The flow created by the impeller forced these fish to swim sustainably. Fish in the resting compartment displayed spontaneous swimming activity. After 18 days of continuous swimming, swimmers had swam an equivalent distance of 1250 km.

**Fish growth**
Mean TL of the START group was 346 ± 6 mm and $U_{\text{opt}}$ for this size class of fish was determined at 2.46 BL s$^{-1}$ (water velocity of 0.85 m s$^{-1}$). After 18 days at $U_{\text{opt}}$ speeds, swimmers had grown an average 39 mm to 385 ± 4 mm whilst resters only grew 21 mm to 367 ± 5 mm; a 92% greater increase in length ($P < 0.05$; Figure 3). Furthermore, swimmers (735 ± 23 g) increased 231 g in BW as compared to the START group (504 ± 27 g), which is a 46% greater increase than the resters (661 ± 32 g) ($P = 0.035$; Figure 3). SGR was 40% higher for swimmers (2.1% BW d$^{-1}$) as compared to resters (1.5% BW d$^{-1}$). Swimmers and resters had similar condition factors ($K = 1.28$ and 1.32, respectively) ($P > 0.05$; Figure 3). Feed conversion ratio was lower and thus more efficient at 1.21 compared to 1.77 in resters.

**Heart and blood**
HW (with BW as cofactor) showed any significant differences between groups (Table 3). Swimmers had a blood flow of 44 ± 3 mL min$^{-1}$, resters 34 ± 3 mL min$^{-1}$ (Table 3). Swimmers thus showed a significantly higher (+31%) cardiac output in the ventral artery ($P = 0.026$; Table 3).

**DISCUSSION**
In this study we have established optimal swimming speeds for juvenile yellowtail kingfish allowing to apply this knowledge for an experimental swim-training trial of 18 days to investigate the effects of swimming exercise on growth performance and cardiac output. Exercise-enhanced growth was robust and not caused by increased feed intake. It may have been caused by increased feeding efficiency indicated by a lower FCR. Doppler ultrasound imaging showed a cardiac output which was significantly higher in exercised fish, no such data have been shown for fish before (to the best of our knowledge).

Plotting polynomial curves for each of the three size classes enabled us to calculate the swimming speeds at which COT was minimal and that correspond to the $U_{\text{opt}}$ (Palstra et al., 2008). Calculating these speeds revealed increasing absolute $U_{\text{opt}}$-values in m s$^{-1}$ with increasing body length which were decreasing when expressed relative to body length (Table 2). As most of the larger fish were still swimming at 1 m s$^{-1}$, polynomial curves predicted by the classical exponential relation between $U$ and MO$_2$ (Jones and Randall, 1978), were not the best fitting curves for groups 2 and 3. Values were however well in line with the two $U_{\text{opt}}$-values known from literature for larger $S. lalandii$: 2.25 BL s$^{-1}$ for yellowtails of 362 mm BL (Brown et al., 2011) and 1.7 BL s$^{-1}$ for yellowtails of 569 mm BL (Clark and Seymour, 2006). By plotting the three $U_{\text{opt}}$-values for juveniles of this study with these
with provides a tool to calculate swimming speed over a size range of 145–569 mm BL and there-
fotma describes the relation between body length and optimal

determine what would be

Figure 1

S. quinqueradiata

also agrees with the deduced highest weight gain in fingerling

respirometric terms (although routine speed was not recorded,

stimulating effects of sustained exercise in active metabolic fish,

Our results support a growing body of work that shows growth-

is predicted to be a result of sustained swimming exercise alone.

Brown et al. speculated on the differ-

tances and given equal feed rations, increased SGR

mated, but recent studies suggest that exercise increases nutrient

for example, exercise increased carbohydrate turnover and promoted protein-uptake in white muscle in gilt-

nality salmonids and pelagic teleosts (reviewed by Davison and

Typically, the enhancement of growth perfor-

mainly salmonids and pelagic teleosts (reviewed by Davison and

nance (e.g., increase in body weight) by exercise is ~40% in

ten species (Davison and Herbert, 2013). Brown et al. (2011)

found exercise-enhanced growth performance in S. lalandi but

only a moderate 10% increase in SGRw in swimmers is predicted to be a result of sustained swimming exercise alone.

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$U_{opt}$ (BL s$^{-1}$) = 234.07(BL)$^{-0.779}$ ($R^2 = 0.9909$; Figure 4). This formula describes the relation between body length and optimal swimming speed over a size range of 145–569 mm BL and there-

with provides a tool to calculate $U_{opt}$-values for fish in this size range.

Eighteen days of sustained exercise training at optimal swim-

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stantially with a 92% gain in length and a 46% gain in body weight as compared to the increase seen in controls. Because resters and

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After 18 days, swimmers ("SWIM, n = 23) showed (A) a significant 92% greater increase in body length (BL) and (B) a significant 46% greater increase in body weight (BW) as compared to resters ("REST, n = 23), while (C) Fulton's condition factor (K) was not different between the two groups. Asterisks above REST-bars indicate a significant difference (∗ P < 0.05; ∗∗ P < 0.01) between the REST-group (n = 10; "time"-effects), while asterisks above SWIM-bars indicate significant differences between swimmers and resters (n = 23; "treatment"-effects).

The START group (n = 10; TL = 346 ± 6 mm, BW = 504 ± 27 g) was sampled before the swim trial commenced. Fish swam ("SWIM, n = 23; TL = 385 ± 4 mm, BW = 735 ± 23 g) at 2.46 BL s⁻¹ or were allowed to perform spontaneous movement ("REST," n = 23; TL = 367 ± 4 mm, BW = 661 ± 32 g) for 18 days. Hearts of 10 fish per group were weighted (HW). Blood flow in the ventral artery (BF) was measured in 13 resters and 13 swimmers. Parameters were statistically tested. Swimmers had a significant (P < 0.05) higher blood flow than resters, detected using pairwise ANCOVA with BW as cofactor and indicated in bold.

When standardized for weight, swimmers in this experiment showed an average blood flow of 59 mL min⁻¹ kg⁻¹, which approximates the results of Clark and Seymour (2006), who measured a blood flow of 50 mL min⁻¹ kg⁻¹ in yellowtails using silastic Doppler flow cuffs. This similarity between the blood flows obtained by an ADV vs. flow cuffs supports the potential of ADVs as alternative method for blood flow measurement, although more comparative studies need to be performed to fully assess the ADV’s potential. The use of ultrasound techniques in teleosts is advancing rapidly: recently Guittreau et al. (2012) used ultrasound imaging to visualize the ovaries of submersed, non-anesthetized, unrestrained catfish. If protocols are optimized to allow the use of ADVs on non-anesthetized, submerged fish, e.g., by using water as a ultrasound transducer medium, the use of ADVs might pose a non-invasive, low-stress alternative to currently used surgical methods.
Vectrino measurements along the x-, y-, and z-axis in the newly designed swim-flume with motor-driven impeller showed a rather uniform flow in the swimming compartment in the applied configuration. When uniformity in flow is lacking, turbulence may have serious impact on the energy expenditure of swimming fish (Liao and Cotet, 2013). For example, fish swimming in vortices downstream of bluff bodies showed reduced muscle activity (Liao et al., 2013), while irregular turbulence has been shown to be detrimental to swimming fish, leading to increased energy expenditure on stability requirements in complex flows (Lupandin et al., 2000; Enders et al., 2003; Liao and Cotet, 2013). In our study, turbulence intensity in the swimming compartment of the flume was 0.08, which is considered low (Lupandin et al., 2000; Pavlov and Skorobogatov, 2009), and should therefore not disturb the optimal swimming economy of the fish in the experimental setup.

The created flow forced fish to swim continuously and in a sustainable way. Fish in the resting compartment showed spontaneous swimming activity, like being performed in tanks at the farm. Vectrino flow measurements in three tanks on the farm showed that fish of 600–1800 g were subjected to flows of 0.19–0.34 m s\(^{-1}\), corresponding to \(\sim 0.62–0.86\) BL s\(^{-1}\). Fish swam generally slightly faster than the flow indicating that the preferred swimming speeds were higher. Still, the preferred swimming speeds for the yellowtails under these conditions were not much higher than 1 BL s\(^{-1}\) and did by far not approach the optimal swimming speeds, contrary to a study on brook charr using a tilted raceway where preferred and optimal swimming speeds were found similar (Tudorache et al., 2011).

CONCLUSIONS
Our study has delivered a formula to calculate optimal swimming speeds for juvenile yellowtail kingfish over a size range of 145–311 mm BL, and together with data from literature (Clark and Seymour, 2006; Brown et al., 2011) up to 569 mm BL: 
\[
U_{\text{opt}} (\text{BL s}^{-1}) = 234.07(\text{BL})^{-0.779} (R^2 = 0.9909). 
\]
The formula was used to execute an experimental scale training experiment of 18 days. Results show that forced sustained exercise at optimal swimming speeds leads to a 92% greater increase in BL and 46% greater increase in BW, an exercise-induced growth stimulation that is much more pronounced than the 10% growth stimulation shown earlier for \(S. lalandi\) by Brown et al. (2011) and that is similar to the optimal growth stimulation for much smaller \(S. quingueradiata\) juveniles (Yogata and Oku, 2000). The applied optimal swimming speed for the experimental fish in this study was 2.46 BL/s (0.85 m/s), much higher than the 0.75 BL s\(^{-1}\) at which Brown et al. (2011) found the highest growth rates, and also higher than the 1.5–2.0 BL s\(^{-1}\) as applied by Yogata and Oku (2000). Moreover, in this study non-swimming and swimming fish were given equal feeding rations (satiation for non-swimmers and restriction for swimmers) so that no differences in feed intake existed between both groups. The difference in growth is reflected by lower FCR for swimmers than for non-swimmers and is thus caused by higher feeding efficiency. It can be expected that when also swimmers are fed until satiation, exercise-enhanced growth will be even more pronounced. Exercise-enhanced growth is accompanied by higher blood flow in the ventral aorta, thus by increased cardiac output, a necessary adaptation in the aerobic phenotype to supply increased oxygen demands.

PERSPECTIVES
The outcomes of this study on experimental scale will need to be validated in an industrial scale setting where several aspects may differ from the experimental situation (densities, feeding conditions, water quality parameters, etc.). One aspect that will be different is that not a swim-flume will be applied but tanks or perhaps raceways as part of RAS having very different hydraulics. It will be challenging to create the flows that are required to induce optimal swimming, especially for the larger sized fish (>500 mm). Flows could be created by using motor-driven impellers like in this study, pumps or gravity, but perhaps a good alternative may be a device that exploits the optomotor response to encourage exercise, like the Optoswim concept (Herbert et al., 2011; reviewed by Herbert, 2013). When validated, implementation of optimal swimming regimes in yellowtail aquaculture, particularly in on-land RAS, will lead to a much faster production cycle. Moreover, increased production by exercise will most probably be accompanied by improved health and welfare aspects.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: http://www.frontiersin.org/10.3389/fphys.2014.00506/abstract

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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