Original Article

Monitoring physical performance and training load in young surf athletes

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ABSTRACT

We aimed to study physical performance and monitor training load (both internal and external) during 12 weeks (3 times per week) and analyse the hypothetical association between physical performance and training load of competitive junior surfers. Twelve competitive surfers voluntarily participated (aged 16.00±1.00y) and completed anthropometric and 8 physical performance tests including weight-bearing dorsiflexion test, functional movement screen, star excursion balance test, squat jump and countermovement jump, sprint & endurance paddling, and breath-hold capacity. Moreover, athletes were monitored by using a heart rate (HR) sensor and global position system (GPS) during each training session (n=36). For internal load (IL), HR, rating of perceived exertion RPE, duration*RPE were used as variables and for external load (EL), duration, total distance, average and maximum speed and pace were considered for analysis. No significant correlations were found between physical performance tests and the training load variables. Significant correlations were found between IL (time*RPE), total distance (r=.58, p<.01), maximum speed (r=.43, p=.04) and duration (r=.60, p<.01). The HR was positively associated with average speed (r=.45, p=.04), pace (r=.43, p=.04), maximum speed (r=.64, p<.01). Total distance, average HR and average speed significantly predicted IL during training F(4.18) =3.17; p =.04; R2 =.48. Data suggests that subjective instruments like RPE seems to be a good instrument to assess the training load in surf training. In terms of training for surfing, maximum speed seems to be a determinant factor in the estimation of IL perception. **Keywords:** Surfboard riding; Internal and external load; GPS; Heart rate, RPE.

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INTRODUCTION

Surfing competition takes place under a variety of conditions that have a significant effect on activity pattern, such as time spent paddling in the prone position, quick stand up and wave riding (O. Farley, Harris, & Kilding, 2012; Méndez-Villanueva et al., 2005). Activity profile studies revealed that paddling and stationary activities represent around 54% to 43% and 28% to 53% of the time, respectively, and only 4% to 6.3% of the time is spent in wave riding (O. R. L. Farley, Harris, & Kilding, 2012; Secomb, Sheppard, & Dascombe, 2015). Such analysis, of both recreational and competitive surfing suggests that surfing can be characterized as a sport requiring multiple short duration and high-intensity bouts of all-out paddling intercalated with relative short paddling (O. Farley et al., 2012). Thus, both technical and physical proficiency are required to succeed in surfing (Fernandez-Gamboa, Yanci, Granados, & Camara, 2017; Secomb, Farley, et al., 2015). Performance analysis incorporates several applications including tactical and technical evaluation, analysis of movement and physical demands (Axel, Crussemeyer, Dean, & Young, 2018; O. R. L. Farley et al., 2012). Nowadays, sport scientists and coaches can obtain objective and quantitative information from athlete's work by heart rate (HR) monitors, evaluate training loads, movement patterns and activity profiles of athletes via global positioning system (GPS) units (Farley, Abbiss, & Sheppard, 2017; O. R. L. Farley et al., 2012; Fernández-Gamboa, Yanci, Granados, Freemyer, & Cámara, 2017).

Both technologies provide a monitoring for the internal (impact of the work on physiological responses) and external load (physical work) of surf athletes in both training and competitive scenarios (Bourdon et al., 2017). Using this type of instrument, the physiological and physical effects of surfing process allow to adjust the training plans and to achieve better conditions for adaptation (Farley et al., 2017; Gabbett et al., 2017). During competitive conditions, internal load (IL) analysis allows to identify that 60% of the surfing time is spent in moderate intensity (54 to 76% HRmax), 19% in light intensity (above 46% HRmax) and only 3% of time is spent above 83% of HRmax (O. Farley et al., 2012). The external load (EL) analysis of a heat of surfing competition showed that 447 m are covered (distance) in which 353.66 m is paddling and 93.85 m in wave riding (Fernández-Gamboa et al., 2017). To our best knowledge, only one study provide information of 2-hour training session of surf athletes covered 6293.2 m with a maximum speed of 35.3 $km.h^{-1}$ and with HRpeak of 171 bpm and HRaverage of 128 bpm (Secomb, Sheppard, et al., 2015). Moreover, a good physical condition of surf athletes contributes to a better performance in both training and competition (O. R. L. Farley et al., 2016). In fact, strong associations were found between relative upper-body pulling strength and sprint paddling time (Sheppard, Walshe, & Coyne, 2012). Junior elite surfers selected to be on a national junior team are stronger in jumping performance, exhibited higher relative lower-body maximum isometric strength and better sprint paddling performance that those not selected (Tran et al., 2015). In youth competitive surfing athletes, the dynamic postural control assumes an important role in performance outcomes and magnitude of lower limb asymmetry (Silva & Clemente, 2017), with trunk stability push-up test from functional movement screen being an important indicator of performance in this specific population (Silva, Clemente, & Lourenco Martins, 2017).

There is currently a lack of studies detailing the physiological demands of competitive surfers during training and free surfing (Farley et al., 2017), with no associations tested between physical fitness levels and training load in surfers during training. This may help to understand the impact of specific training programs in performance outcomes.

The purpose of this study was i) to assess the physical performance through field and swimming pool-based tests, ii) monitoring internal and external load through HR and GPS during 12 weeks, and iii) analyse the association between physical performance and both internal and external loads in competitive junior surfers.

METHODS

Experimental approach to the problem

In the present study, a team of competitive junior surfers was followed for 12 weeks. Athletes were monitored with an HR sensor (Polar Electro, Kempele, Finland) and a global position analysis (GPS) (Polar V800, Polar Electro, Kempele, Finland) during each training session (n=36).

Physical tests were conducted in one day at Surfing Viana High Performance Centre and in a swimming pool. All athletes were abstained from intensive exercise, surf training or any form of extreme physical efforts in the 48-hours period before testing. Before the tests, athletes performed a standardised warm-up, consisting of 10 min of jogging at a self-selected pace and dynamic mobility movements. Whereas, before swimming pool tests, the warm-up consists of 10 min of freestyle stroke at a self-selected pace and 100 m of paddling on the surfboard with 4 of 5 m sprints in every 25 m. Then, participants completed the laboratory physical tests in the following order: i) weight-bearing dorsiflexion test (WB-DF), ii) functional movement screen (FMS), iii) star excursion balance test (SEBT), iv) squat jump (CJ), v) countermovement jump (CMJ) and the swimming tests: vi) endurance and sprint paddling tests and vii) breath-hold capacity.

Participants

Twelve junior surfers (6 female and 6 males; age 16.00±1.00y; body mass index 20.88±1.95) from national and regional surf competitions volunteered participated in the current 12-wk study (Table 1). The athletes trained 3 times *per* week throughout the pre-championship period. The athletes have regular stance on the surfboard (left feet in front). If the athlete had an acute injury during the previous 30 days that prohibited them from full participation in regular training or competition was excluded from the study. All subjects and parents for those under 18 years received a written informed after a clear verbal explanation of the experimental design and potential risks and benefits of the study. The study was approved by the local institutional ethical review board and was conducted in accordance with the provisions of the Declaration of Helsinki.

Table 1. Characteristics of surf athletes.

	Total	**Male**	**Female**
Age (years)	16.00±1.00	16.17 ± 1.07	15.83 ± 0.90
Height (cm)	166.65±8.25	173.15 ± 6.23	160.15 ± 3.57
Weight (kg)	58.15±8.19	63.42 ± 8.07	52.88 ± 3.65
BMI	20.88±1.95	21.10±2.06	20.65±1.81
Body fat (%)	18.23±8.10	12.40 ± 6.01	24.05 ± 5.21
Lean Mass (%)	38.03±5.38	43.02 ± 2.01	33.05 ± 2.04

Data was expressed as mean ± SD; cm, centimetres; kg, kilograms; BMI, Body Mass Index; %, percentage.

Anthropometrics

Body weight, percentage of body fat and lean mass were assessed by an Omron Body Composition Monitor BF511 researches (Omron Healthcare Co. Ltd., Japan), a 8-sensor, one-frequency (50 kHz, 500 uA) bioelectrical body impedance analysis device, under strictly standardized conditions as set by the manufacturer and previous research to the nearest 0.1 kg (Pietiläinen et al., 2013). The height of the participants was evaluated to the nearest 0.1 cm with a portable stadiometer (SECA 217, Germany). All participants were dress light clothing and stood barefoot, with the head oriented according to the Frankfurt plane, for accessing the height and according to the manufacturer specification when using the body composition monitor.

Physical performance testing

Weight-bearing dorsiflexion (WB-DF) test: ankle dorsiflexion range of motion (ROM) was evaluated through weight-bearing lunge using the Leg Motion System (L*egMotion, Check your Motion, Albacete, Spain) (Calatayud et al., 2015).* This portable device allows an easier completion weight-bearing lunge test has been shown high reliability and validity for surf athletes, as previously reported (Lundgren et al., 2013). Subjects were in a standard position on Leg Motion System with the assigned foot on the measurement scale while the opposite foot was positioned out of the platform. While maintain this position, subjects were instructed to perform a lunge in which the knee was flexed with the goal of making contact between the anterior knee and metal stick. When subject was able to maintain heel and knee contact, the metal stick was progressed away from knee. Maximum dorsiflexion ROM during the Leg Motion System test was defined as the maximum distance of the toe from the metal stick while maintaining contact between the stick and knee for three seconds without lifting the heel. All the measurements were completed with the participant barefoot; first performing all tests with assigned foot and then with the contralateral foot in a counterbalanced order. Three trials were allowed for each side with 10 sec of passive recovery, and the average value of the three trials was used for data analysis.

Functional Movement Screen (FMS)

Comprises seven fundamental movement tasks and three clearance tests. Each pattern from FMS was assessed according to the general procedures and recommendations previously described (Cook, Burton, Hoogenboom, & Voight, 2014). An FMS specialist with 3 years of experience conducted the tests with an official FMS test kit. To each patterns were assigned a score of 0 to 3, represented, according to the relevant criteria: 0 – pain anywhere in the body; 1 – unable to complete the movement pattern or is unable to assume the position to perform the movement; 2 - able to complete the movement but must compensate in some way to perform the fundamental movement; 3 - performs the movement correctly without any compensation, complying with standard movement expectations associated with each test. Approximately 10 sec of rest was provided between trials and one minute between tests. The best scores of the seven tests for each athlete were kept in the analysis and calculated a composite score.

Star Excursion Balance Test (SEBT)

Is a dynamic test that requires neuromuscular characteristics such as strength, flexibility and proprioception, commonly used to assess physical performance and screen deficits in dynamic postural control due to musculoskeletal injuries (e.g. chronic ankle instability), to identify athletes at greater risk for lower extremity injury (Gribble, Hertel, & Plisky, 2012). The dynamic postural control was assessed by using the OctoBalance device *(OctoBalance, Check your Motion, Albacete, Spain).* This measurement system is based on an octagon-shaped platform, which is magnetised in each direction with an extending measuring tape. Each trial consists of pushing the marked point, situated at the top of the measuring tape, preferentially with the toes as far as possible in the designated direction. A modified version of the SEBT was applied where the subjects only perform anterior reach (SEBT-A), posterolateral reach (SEBT-PL) and posteromedial (SEBT-PM) reach (Gonzalo-Skok, Serna, Rhea, & Marín, 2015) of the 8 possible directions. Before the assessment of each trial, the measuring tape was placed at a minimum distance of 30 cm to start the excursion. Each trial was validated by a visual inspection to ensure that the athlete remains their heel on the anterior-posterior line on the platform without putting the toes on the marked point. During the test, the athletes were instructed to maintain their hands on the hips and stayed barefoot. Three trials were allowed for each side with 10 sec of passive recovery, and the average value of the three trials in each leg was used for data analysis.

Squat Jump (SJ) and Countermovement Jump (CMJ)
For testing performance of lower extremity muscle power and strength, all athletes performed both SJ and CMJ tests. Both were conducted in a contact platform (Chronojump-Bosco system, Spain), connected to a personal computer with the Chonopic microcontroller and register the jumps in the Chronojump software (version 1.6.0 for Windows). All subjects were instructed according to the tests procedures and requirement. The Bosco System is a validated (Blas, Pedullés, Lopez del Amo, & Guerra-Balic, 2012) and accurate (Pueo, Lipinska, Jiménez-Olmedo, Zmijewski, & Hopkins, 2017) device for measuring vertical jump performance. In the SJ test, athletes maintained a static position with a 90º knee flexion for 2 sec before each jump attempt, without any preparatory movement and keeping hands on hips. Whereas CMJ test begin from an erect position and use quick crouching action followed immediately by a vertical jump as high as possible, landing back on the mat. Three attempts were assessed with a 15 sec rest interval between each trial and 2 min before each test. The best score was considered for further analysis.

Sprint and Endurance Paddling tests
Sprint-paddle testing was conducted in an indoor 25-m swimming pool, accordingly with previous researches (O. R. L. Farley et al., 2013; Secomb et al., 2013). Each subject performed the test on the same size surfboard and wore surfing board shorts. All subjects performed a 3 maximum-effort sprint-paddling time trials (i.e., 3 x 10 m) to determine maximum sprint-paddling performance. The sprint-paddle efforts were initiated from a stationary, prone lying, floating position. To determine the maximum sprint paddling performance the best of the three trials was considered. The 10 m course were clearly identified with a different coloured buoy. Each trial was timed using two assistants, one controlling the start position and other at the end of the course that was responsible for controlling the time and for the acoustic sound of start. To carry out a correct and precise testing process, only the second assistants have a stopwatch and a whistle.

The timed endurance-paddle test was conducted over a 20-m up-and-back course in the same pool, using small buoy markers at both ends of the 20 m distance, so that continuous paddling to a total of 200 m could be accomplished. Thus, subjects paddled 20 m and completed a turn at each end around the buoy until the 400 m was completed. The subjects paddled up to around the buoy completing 180º turn while remaining prone on their surfboards. The time to complete the endurance-paddle test allowed for determination of subjects' maximum aerobic speed, which was intended to reflect their endurance capabilities in the specific context of surfboard paddling.

Breath-hold capacity
To determine maximum time under water without any respiratory assistance, all subjects performed 2 trials with 2 min rest before each attempt. The breath-hold capacity test was initiated from a stationary position (holding a support wedge of the swimming pool) with the feet on the swimming pool bottom at an average height of 90 cm. The surrounding area (2x2 m) were clearly from objects and persons. Each trial was timed using one assistant who was responsible for controlling the time and for performing the countdown (3, 2, 1, start). The timer was started after the mouth and nose be under water and stopped when the participant backs out of the water with the mouth and/or nose. The best score from the 2 trials (maximum time under water) was considered for further analysis.

Internal and external load
For controlling the internal load (IL), a Polar V800 HR Monitor with a Polar H7 chest strap (Polar Electro, Kempele, Finland) and the Rating of Perceived Extension (RPE) were used during and after the surf training, respectively. The Polar V800 is valid to detect RR intervals with an error of .09% and an intra-class correlation coefficient of >0.99 (Giles, Draper, & Neil, 2016) and the RPE have been shown to be a useful tool for

determining exercise intensity, as it is related to physiological indicators of exercise stress, including lactate concentration and HR (Chen, Fan, & Moe, 2002). The Borg CR-10 category-ratio scale was used for the RPE (Borg, 1998). To improve the consistency of the measurement, all athletes were previously familiarized with the use of the RPE by training with a graphical, coloured, verbal-anchored scale shown in previous physical training, to disclose the learning effects (Psycharakis, 2011). The IL was calculated as a product of training session time multiplied by RPE (time*RPE). The EL was measured by the motion tracker of the Polar V800 GPS that collected position data at a frequency of 2.4 Hz sampling rate with an integrated accelerometer that collects accurate data (Hernández-Vicente, Santos-Lozano, De Cocker, & Garatachea, 2016) and has been used in surfers (Fernández-Gamboa et al., 2017). The athletes received the motion trackers before the warm-up to get familiarized and to fixing the GPS signal avoiding inaccuracy in the data. The weather conditions during the testing days were optimal (bright air, open field), which maximized the GPS reception. The motion trackers were fully charged, synchronized with the individual data of the athlete (age, weight, height and training level) and in the self-selected wrist. The Polar V800 was started immediately after the surfer enters the water and stopped when exiting the water with an automatic record of the RPE from the training. All the data were uploaded post-experimentally to the Polar Flow App and download to a .CSV file and the duration, total distance, average and maximum of speed and pace during each training sessions were considered for analysis.

Statistical analyses

Data was expressed as mean ± SD. Assumption of normality was verified using the Shapiro-Wilk test. Pearson's correlation coefficients were used between physical tests data and IL – HR, RPE and time*RPE, and EL - duration, total distance, average and maximum of speed and pace of training sessions. The following correlation scale was adopted: trivial ($r < 0.1$); small ($0.1 \leq r < 0.3$); moderate ($0.3 \leq r < 0.5$); large ($0.5 \leq r < 0.7$); very large ($0.7 \leq r < 0.9$); and nearly perfect (≥ 0.9) (Hopkins, Hopkins, & Glass, 1996). A multiple regression analysis was used to predict IL based on the values of EL. Collinearity statistics was tested before the final model. A threshold of 2.5 of variance inflation factor was defined to exclude manage variables and to avoid multicollinearity. All data sets were tested for each statistical technique and corresponding assumptions and performed using SPSS software (version 24.0, Chicago, Illinois, USA). Statistical significance was set at 5%.

RESULTS

The table 2 and 3 show the mean ± SD of physical performance tests and training load variables, respectively. No significant correlations were found between physical tests and the training load variables.

Significant correlations were found between IL and EL variables. Large correlations were found between IL (product between time*RPE) and the total distance ($r=.58$; $p=.01$). Moderate correlations were found between IL and the maximum speed ($r=.44$; $p=.04$). Maximum HR was moderately correlated with average speed ($r=.45$; $p=.04$) and pace ($r=.43$; $p=.04$) and largely correlated with maximum speed ($r=.64$; $p< .01$). Both IL ($r=.60$; $p <.01$) and total distance ($r=.79$; $p< .001$) were largely correlated with duration of the training session (Figure 1).

A multilinear regression analysis was conducted to test the variance of IL during training sessions in dependency of total distance, average HR and average speed. These variables significantly predicted IL during the training session, $F(4.18) = 3.17$; $p = .04$; $R2 = .48$. The unstandardized coefficient, B1, for total distance is equal to .063, for average HR is -2.06, for average speed, is -37.84 and for pace 1.23. The standardized coefficients (Beta) was 0.68 for total distance, -.26 for average HR, -.35 for average speed and .19 for pace.

Table 2. Physical performance of surf athletes.

	Total
Ankle dorsiflexion ROM	
WB-DF right	12.27±2.12
WB-DF left	11.45±1.34
FMS	
Total Score	15.20±3.25
SEBT	
SEBT-A Right	51.10±3.31
SEBT-PL Right	69.80±5.49
SEBT-PM Right	72.40±15.68
SEBT-A Left	54.70±5.02
SEBT-PL Left	68.30±7.37
SEBT-PM Left	76.60±9.75
Lower extremity power and strength	
SJ (cm)	30.18±6.31
CMJ (cm)	30.20±6.33
Sprint and Endurance Paddling	
10-m sprint paddling (sec)	7.23±.35
200-m paddling (min)	3.77±.51
Apnea (sec)	72.50±15.17

Data was expressed as mean ± SD. FMS, Functional Movement Screen; CMJ, Countermovement Jump; cm, centimetres; sec, seconds; min, minutes; SEBT-A, Star Excursion Balance Test anterior reach; SEBT-PL, Star Excursion Balance Test posterolateral reach; SEBT-PM, Star Excursion Balance Test posteromedial.

Table 3. Internal and external load variables monitored during 12-wks

	Total
Internal Load	
IL (duration*RPE, A.U.)	220.25±50.14
Maximum HR (bpm)	176.13±7.38
Average HR (bpm)	145.75±7.85
RPE	5.81±1.15
External Load	
Duration (min)	46.07±5.23
Total distance (m)	3188.75±402.83
Average Speed ($km.h^{-1}$)	4.16±.45
Maximum Speed ($km.h^{-1}$)	14.94±2.52
Average Pace ($m.min^{-1}$)	69.48±7.50

Data was expressed as mean ± SD. A.U., Arbitrary unit; bpm, beats per minute; RPE, rating of perceived exertion; min, minutes; m, meters; $km.h^{-1}$, kilometre per hour; $m.min^{-1}$, meter per minute.

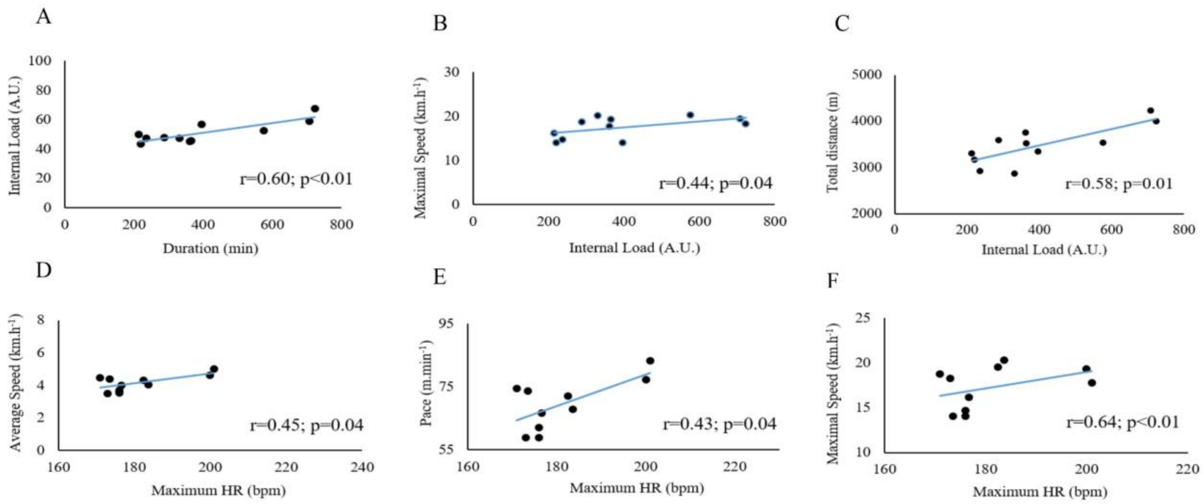

Figure 1. Scatter plot for the relationship between IL, total distance (A), and maximum speed (B), and duration (C). The maximum HR, average speed (D), pace (E) and maximum speed (F).

DISCUSSION

Considering the purpose of the present study, it was found that RPE, total distance, maximum speed and session duration are very important indicators when monitoring a surf training. Analysing the data, and even with no significant correlations between physical performance and the training load variables, there were large to moderate correlation between internal and external variables. To the best of our knowledge, just one study (Fernández-Gamboa et al., 2017) report the RPE after a surf competition but no previous research has reported after training session. These significant correlations lead to some important indications. Aerobic conditioning is directly linked to the physical capacity to catch as many waves as possible during surfing (O. Farley et al., 2012) leading to the expected significant large correlation between IL and total distance. Because IL is the product of time*RPE and when the total distance increases the RPE and the total time also increased, RPE after a surfing training can be an important indication to control training load, even without the possibility of monitoring the total distance. So, if during a 2 hours surf session about 1 hour is paddling (Secomb, Sheppard, et al., 2015) the resultant RPE indirectly represent the amount of total time and distance of the training. This subjective method was also stated to be a good instrument to assess the heat load of a surf competition (Fernández-Gamboa et al., 2017). In the same perspective and taking in consideration that maximum speed is obtained during wave riding (O. R. L. Farley et al., 2012) and there is a very large significant relationship between wave riding distance and respiratory RPE during a competition heat load (Fernández-Gamboa et al., 2017), is evident that wave sizes can be a determinant in the RPE and even without changing the total distance and total time significant influence the IL. This statement has more strength since Barlow et al 2014 concludes that wave parameters have significant effects on the physiological response and performance characteristics of surfing including HR. The average HR, 145.75±7.85 bpm is similar to that found in the study of Barlow et al (2014) (146.4 ± 16.8 bpm), Mendez-Villanueva et al (2005) (146.20 ± 20.0 bpm) and Farley et al (2012) (139.7 ± 11.0 bpm). Our study found exactly a significant positive correlation with maximum HR and maximum speed, confirming previous observations and strengthening the relationship between the short duration of the time spent riding the wave (O. R. L. Farley et al., 2012; Secomb, Sheppard, et al., 2015) and the necessity to performing manoeuvres. As expected, IL and total distance were significantly correlated with duration of training session, supported by the increasing volume. In fact, 48% of the variance of IL during training sessions can be attributed to the total distance, average HR and average speed (F(4.18) = 3.17; p = .04; R2 = .48). The association between maximum HR, average speed and pace found in the present study is related mainly to time spent in a low speed zone and work rates below the lactate threshold (Barlow, Gresty, Findlay, Cooke, & Davidson, 2014). Furthermore, the short bouts of time spent in wave riding, the long paddling time back to the break (Fernández-Gamboa et al., 2017), paddling to reposition in the take-off area and perform a powerful strokes to position the board in the right location and gain enough momentum to catch waves (Mendez-Villanueva, Bishop, & Hamer, 2006) are factors that strongly contribute to changes in speed and pace that all so influence maximum HR.

One of the main purposes of the present study was to analyse the hypothetical association between physical tests and IL and EL variables, but unexpectedly no correlation was found. This finding may be explained, in part, by the fact that training outcomes are ultimately the interplay of many variables across the training process (Impellizzeri, Rampinini, & Marcora, 2005) and the physiological responses to activities like paddling, wave riding or sprint paddling change with surf training (Farley et al., 2017). Moreover, the physical performance tests used in the present study are more related with the magnitude of lower extremity asymmetry and postural control than surf training requirements (Silva & Clemente, 2017). Besides the limitation inherent of the sample size and the motion tracker used, the current data provide some useful strategies for surf coaches during training. Future studies should focus on controlling IL and EL for the same athlete from a longer period with the controlling of wave parameters and surfer’s perception of the wave conditions.

CONCLUSION

Total distance, maximum speed, session duration and RPE are determinants factors when monitoring surf training, with maximum speed being a determinant factor in the estimation of IL. The subjective instruments like Borg CR-10 category-ratio scale seem to be a good instrument to assess the training load during surf training.

PRATICAL APPLICATIONS

The reliable, accessible and easy to administrate method like Borg CR-10 category-ratio scale seems to be a good instrument to assess the training load of a surfing training, giving an important indication of the IL. During training routines, this scale allows surfers and coaches to quantify workloads and to compare data between different conditions and from other devices as HR monitor or GPS. The proportion of total distance, total time and maximum speed seem to have an important influence in the IL during surf training, providing adjustable variables to modifying training stimulus.

AUTHOR CONTRIBUTIONS

BS, GC, and FMC conceived and designed the experiments; BS and GC performed the experiments; BS and SRG analysed the data; BS, GC, SRG and FMC wrote the paper and approve the final submission.

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DISCLOSURE STATEMENT

There were no conflicts of interest.

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