RESEARCH ARTICLE

Repeatability of escape response performance in the queen scallop, *Aequipecten opercularis*

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SUMMARY

In order for natural selection to operate, physiological and behavioural traits must exhibit both inter-individual variability and intra-individual consistency (i.e. repeatability) in performance. In this study, we describe individual variation and temporal repeatability in the escape responses of the queen scallop, *Aequipecten opercularis*, and determine whether individuals exhibited consistently high or low rankings in different aspects of the escape response. Five measures of individual performance were recorded on four occasions (days 0, 2, 7 and 28), providing proxies for sensory acuity (response latency), immediate and sustained swimming performance (burst and average clap rates), and swimming endurance (total number of claps and total time spent clapping). All components of the escape response exhibited significant inter-individual variability (all *P*<0.0001). Escape response latency, burst clap rate, total number of claps and total duration spent clapping maintained significant repeatability over 28 days (all *P*<0.016). Average clap rate was repeatable in the short term (2 days, *P*<0.0001) but repeatability declined by 28 days (*P*=0.097). Concordance analysis indicated that individuals maintained the same performance rankings over time for each component of the escape response (all *P*<0.001). In addition, some individuals ranked as consistently high or low performers across response latency, burst and average clap rate, and total number of claps. An individual's ability to evade predators through the provision of an escape response of an appropriate magnitude, subject to physiological, behavioural and organismal constraints, will have clear fitness-related consequences.

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INTRODUCTION

The process of natural selection has provided one of the most fundamental conceptual tools in biology for understanding the processes of phenotypic plasticity, speciation and biodiversity (Nespolo, 2003). However, natural selection will only act on individual traits that display consistent inter-individual variability, have a contributory genetic component and confer superior fitness (i.e. increase the chance of survival and reproduction), subject to phenotypic plasticity and environmental variability (Bennett, 1987; Nespolo, 2003). In order for natural selection to operate on traits and select between individuals, the physiological or behavioural performance of individuals must be consistent (i.e. repeatable) over time compared with the performance of others (Bennett, 1987).

To date, the majority of studies quantifying repeatability of individual physiological performance have examined metabolic rate (reviewed in Nespolo and Franco, 2007) or locomotor performance (see below) as these two physiological traits can be measured easily and non-terminally, and have clear fitness-related consequences with regards to energy allocation, predator evasion and food capture. However, repeatability in other physiological traits such as evaporative water loss (e.g. Chown et al., 2006; Versteegh et al., 2008) and blood chemistry parameters (e.g. Ford and Paillard, 2007; Tieleman et al., 2010) have also been examined. The repeatability of inter-individual locomotor performance has been well studied in vertebrates: fishes (e.g. Kolok, 1999; Oufiero and Garland, 2009), amphibians (e.g. Austin and Shaffer, 1992; Watkins, 1997), reptiles

(e.g. van Berkum et al., 1989; Bonine and Garland, 1999; Elnitsky and Claussen, 2006) and birds (Renner, 2006). These studies have revealed that not only does locomotor performance vary significantly amongst individuals within a species but also the degree of inter-individual variability in performance is often consistent with repeated measures over time, although the degree of repeatability declines with increasing time interval between the repeated measures (e.g. van Berkum et al., 1989; Austin and Shaffer, 1992; Oufiero and Garland, 2009). In contrast, however, quantification of the repeatability of locomotor performance of invertebrates is extremely limited (e.g. Clusella-Trullas et al., 2010; Prenter et al., 2010; Brokordt et al., 2012).

Repeatability of physiological performance over time has been quantified using either intra-class correlation (Lessells and Boag, 1987) or Pearson's product-moment correlation (i.e. inter-class correlation) (Hayes and Jenkins, 1997). Both approaches determine the level of statistical significance and calculate a coefficient between 0 and 1 indicating the degree of repeatability (1 is equal to perfect repeatability). Intra-class correlation can be used where several repeated measures have been made over time and where either there is no significant increase in animal size or the average value of the physiological trait being measured over the time interval between repeat measures (e.g. Fuiman and Cowan Jr, 2003) or the data have been size-corrected prior to analysis (e.g. Chappell et al., 1995). Inter-class correlation (Pearson's *r*) is used where animals have increased in size, there has been a shift in the average value

of the physiological trait over time or when only two measures of performance are being compared (e.g. McCarthy, 2000).

In terms of abundance, functional ecology, distribution and adaptation to varying habitats, bivalve molluscs have been demonstrated to be a remarkable evolutionary success, often representing dominant or important groups in most benthic ecosystems (Barnes et al., 1999). In addition, the Pectinidae (scallops) are unusual within bivalves in having evolved extended locomotor function in the form of swimming, known to facilitate predator evasion (e.g. Moore and Trueman, 1971; Pérez et al., 2008). Swimming is achieved by propelling water dorsally out either side of the hinge through repeated adductions of the valves (Moore and Trueman, 1971). The closing of the valves is performed by the adductor muscle, antagonistic to an elastic hinge ligament (Bailey and Johnston, 2005). The adductor may be divided into two regions; the smooth-fibre tonic muscle region is used for ventilation of the mantle during periods of resting, whilst the larger striated-fibre phasic muscle region, which can repeatedly and rapidly contract, is exclusively used during the escape response (Fleury et al., 2005; Pérez et al., 2008). Because of the ease with which the escape response can be recorded, we have learnt much about the muscle physiology underlying the contractile and recovery phases of the escape response and how the magnitude of the escape response relates to parameters such as stress (Jenkins and Brand, 2001), age/size (Philipp et al., 2008; Schmidt et al., 2008; Labrecque and Guderley, 2011), temperature (Guderley et al., 2009), reproductive state (Brokordt et al., 2000a; Brokordt et al., 2000b; Kraffe et al., 2008; Pérez et al., 2009), genotype (Pérez et al., 2011) and diet (Guderley et al., 2011). As a well-studied taxa, individual differences in scallop escape response performance have been reported and repeat measures of known individuals compared over time (using paired t-tests or repeated measures ANOVA depending on the number of repeat measures being compared) have enabled the degree to which individual performance is consistent over time to be examined (e.g. Fleury et al., 2005; Guderley et al., 2009; Pérez et al., 2009; Guderley et al., 2011). However, the degree of individual repeatability in escape response performance in scallops using the correlative approaches described above has only recently been conducted for repeat measures made 5 min apart (Brokordt et al., 2012) and the longer term repeatability over time has not been examined.

The purpose of the present study was to (1) describe the inter-individual variability in the escape responses of the queen scallop, *Aequipecten opercularis* Linneaus 1758, induced by a predatory threat, (2) determine the repeatability of individual escape responses over the time scale of days–weeks by quantifying the repeatability of performance measures and the consistency of individual ranking based on performance within the group, and (3) determine whether different aspects of the escape response co-varied, i.e. whether some individuals exhibited superior performance in all aspects of the escape response and could be considered natural 'athletes'.

MATERIALS AND METHODS
Animal collection and husbandry

Scallops (*ca*. 150, shell height *ca*. 60–70 mm), in good visible condition, were collected by Newhaven scallop dredge from the Isle of Man and transferred to the School of Ocean Sciences, Bangor University, where they were held in tanks supplied with continuous flow-through unfiltered seawater (temperature range 15.1–16.2°C) providing a natural supply of algae. In addition, scallops were fed with supplementary cultured algae after experimentation on the days when scallops were exercised up to their anaerobic capacity (see

below) to promote full recovery from exhaustive exercise. Prior to experimentation, the scallops were acclimated for 2 weeks and during this period dead and visibly damaged scallops or those displaying atypical behaviour upon handling were removed. Starfish *Asterias rubens* Linnaeus 1758 (*N*=15, diameter 10–18 cm) were collected by hand during low tide from the Menai Strait, maintained in adjacent tanks to the scallop stock tanks independently supplied with seawater from the same flow-through system and fed one mussel per individual per week during scallop acclimation and experimentation.

Experimental set-up

A previously published method (Jenkins and Brand, 2001) was used to record valve adductions in immobilised *A. opercularis* using high-speed filming. Ten days before the start of the experiment, scallops were marked on the upper (left) valve to enable individual recognition, measured (shell height ±0.1 mm, S_{h1}, Table 1) and prepared for the experiment by attaching a nylon bolt to their lower (right) valve using Araldite rapid-drying epoxy resin. Reciprocal nuts were attached to six house bricks using putty-type epoxy cement (Milliput Standard) (Jenkins and Brand, 2001). Only scallops that showed no visible deterioration in condition and exhibited normal resting and handling behaviour during the acclimation period were deemed usable in the repeatability experiments (*N*=41).

During each trial, a brick was placed in each of six transparent, conditioned plastic tanks (L×W×D, 32×20×20 cm) filled with 10 l seawater and supplied with a diffuse air supply. After 15 min aeration, scallops were selected at random and quickly transferred and screwed onto each brick underwater to minimise handling and emersion stress (Jenkins and Brand, 2001). Scallops were given a further 15 min to resume natural resting behaviour, i.e. to adopt a gape of ~30 deg to aerate gills and filter feed (Fleury et al., 2005). A Casio Exilim EX-FH100 high-speed (240 frames s^{-1}) camera was

Table 1. Summary of body metrics for the queen scallops (*Aequipecten opercularis*) used in the escape response performance measurements

Body metric	Mean ± s.d.	Range
Initial shell height (S_{h1}, cm)	65.1±4.6	52.4–75.5
Final shell height (S_{h2}, cm)	65.0±4.6	52.3–75.9
Soft body wet mass (g)	14.32±3.19	8.43–21.91
Soft body dry mass (g)	2.58±0.75	1.30–3.96
Water content (%)	82.2±2.1	78.0–86.2
Shell dry mass (g)	24.69±4.78	13.3–40.2
Gonad wet mass (g)	0.86±0.45	0.21–1.97
Adductor wet mass (g)	6.10±1.76	2.91–9.51
Muscle index (%)	42.1±5.3	27.8–50.5
Condition index (%)	10.5±2.9	5.4–16.5
Gonadosomatic index (%)	15.2±9.2	3.6–41.4
Stage 1 (*N*=4)*	6.1±2.1	3.6–8.9
Stage 2 (*N*=3)	11.3±0.6	10.9–11.7
Stage 3 (*N*=12)	13.4±7.6	4.6–31.6
Stage 4 (*N*=17)	17.2±10.3	6.4–41.4
Stage 4.5 (*N*=1)	21.5	–
Stage 5 (*N*=4)	24.3±6.7	15.6–30.6

Data are presented for *N*=41 scallops. Data for gonadosomatic index are presented for each gonad stage and as an overall mean value.

*Gonad stages are based on the assessment scale of Duinker and Nylund (2002). The equations for calculating the morphometric indices are presented in Materials and methods.

then positioned in front of each tank in turn so that it had a level 'side-view' of the shell margin to capture the escape response of each individual. During filming, each tank was illuminated by a DC-powered waterproof LED light source, to reduce flicker caused by overhead lighting (~50 Hz). Once recording had begun, the escape response was initiated by touching the scallop's mantle tentacles proximal to the hinge with the tube feet of *A. rubens*, a known predator (Jenkins and Brand, 2001; Bailey and Johnston, 2005). The escape response consisted of a number of phases each comprising a series of sequential claps (termed phasic contractions), where the valves were rapidly opened above the 30 deg resting gape and rapidly closed (i.e. a 'clap'), followed by a short resting period where the valves where maintained at the resting gape or were opened and closed very slowly (termed tonic contractions). A different starfish was used for each group of six scallops. The instance of contact (stimulus) was indicated on the video for post-experimental video analysis. Disturbance before or during the escape response trial was kept to a minimum to avoid premature responses or influencing the escape response once initiated. On the rare occasion a scallop responded prior to stimulation, the trial was delayed for a further 15 min. The starfish tube feet were left in place to provide a continuous stimulus throughout the trial (Jenkins and Brand, 2001), taking care to prevent the tube feet from attaching to the scallop shell (Bailey and Johnston, 2005). The trial was terminated when either the valves closed firmly or there was no response during 60 s of continuous contact (Jenkins and Brand, 2001). After testing, scallops were returned to the holding tanks, the experimental tanks were rinsed and the seawater replaced to remove the influence of residual chemical traces from the previous trial, and another group of scallops was introduced into the test aquaria. Jenkins and Brand recommend that a minimum of four sequential claps be initially performed to qualify as a 'swimming' escape response (Jenkins and Brand, 2001). Based on this rule, swimming responses were observed in 100% of trials run during the experiment. Queen scallops show 100% recovery from sustained escape responses after ~1–1½ days (Bailey et al., 2003). Therefore, repeatability trials examining escape responses were conducted 2, 7 and 28 days following their first trial (*t*=0).

Following the 28 day trials, all scallops were re-measured (S_{h2}, Table 1) and dissected to obtain the following measurements. Gonad maturity was assessed by visual inspection using a published scale (see Duinker and Nylund, 2002). The wet mass (±0.01 g) of the adductor muscle, gonad and all remaining tissues was recorded and the dry mass of the shell and the combined soft body dry mass were determined by drying to constant mass at 55°C. The following indices were calculated to assess the condition of each scallop: gonadosomatic index [GSI (%), (100×gonad mass)/soft body wet mass], muscle index [MI (%), (100×adductor muscle mass)/soft body wet mass] and body condition index [CI (%), (100×soft body dry mass)/shell dry mass] (Schmidt et al., 2008).

Post-experimental analysis

The high-speed video files were analysed in Windows Moviemaker, v. 5.1, which permitted frame-by-frame analysis of scallop escape responses and the real-time timings of various events reported relative to the initiation of the stimulus. The following performance components were calculated or recorded from each video: (1) response latency (±0.01 s), defined as the time between the moment of application of the stimulus and the start of the first valve adduction, (2) the total number of claps, defined as the total number of valve adductions that took place during periods of phasic contraction in the experimental trial (Fleury et al., 2005), (3) total clapping duration

(±0.01 s), defined as the total time that passed during all periods of phasic contraction (that is, the duration of activity following the first valve adduction but excluding periods of tonic contraction, i.e. the resting periods), (4) burst clap rate (claps s^{-1}), defined as the greatest valve adduction rate attained, which usually occurred during the initial 'swimming' response (Jenkins and Brand, 2001), i.e. the first instance where four or more adductions occurred without being separated by a period of tonic contraction, and (5) average clap rate (claps s^{-1}), calculated as the total number of claps divided by the total clapping duration. Thus, this study reports five measures of individual performance providing proxies for sensory acuity (response latency), maximum and sustained swimming performance (burst and average clap rates) and swimming endurance (total number of claps and total time spent clapping).

Statistical analysis

In our study, we chose to only use individuals for which data were recorded in all four trials, i.e. 36/41 scallops were assessed as a result of two failed recordings and three instances where individuals broke free of their restraint during one of the escape response trials. In addition, three individuals were removed from the latency analyses because of marginally premature responses only identified during video analysis.

Prior to analysing data for inter-individual variability and individual repeatability, size dependency in performance was assessed. We compared initial and final shell heights to test for a significant increase in size. This was taken as the most robust measure of size as attempts to measure total mass (i.e. shell plus body tissue) were confounded by variable water retention within the mantle cavity (data not shown). Initial and final shell heights (S_{h1} and S_{h2}, Table 1) were not significantly different (paired *t*-test, $t_{2,40}$=1.13, *P*=0.26), reducing the likelihood of size-related effects on the performance within individuals during the 28 day experiment, though this measure of size does not provide information on a change in visceral or gonad mass. We also examined whether differences in performance between individuals were (at least in part) a function of allometry (Bonine and Garland, 1999; Peterson and Husak, 2006) by regressing individual performance measures against shell height (S_{h1} for data from days 0, 2 and 7, and S_{h2} for data from day 28) after confirming normality and homoscedasticity for the performance measures and shell height. Significant size dependency was observed for total clapping duration on days 0 and 7, burst clap rate on day 28 and average clap rate on days 7 and 28. Where a significant size dependency was identified in any of the four trials for a given performance measure, all the data for that performance measure (i.e. all four trials) were size corrected as described elsewhere (Reidy et al. 2000). This was achieved by adding the trial-specific regression residual for each individual to the group mean value for that trial to create size-corrected values for each individual whilst retaining units of measure. Only in situations where no significant size trends were identified for any trial of a performance measure did the measures for all trials remain uncorrected. Thus, uncorrected data for response latency and total number of claps, and size-corrected data for clapping duration and burst and average clap rate were used in all subsequent analyses. All data distributions were normal and homoscedastic following log$_{10}$ transformation except burst clap rate. Inter-individual variation in performance measures was analysed using one-way ANOVA (or Kruskal–Wallis for burst clap rate data), identifying each individual as a separate treatment with the four repeat measures of performance as replicate samples.

The repeatability and consistency over time of an individual's escape response performance were examined in three different

ways. Pearson's product-moment correlation coefficients (r) were calculated to assess the repeatability of initial performance in each subsequent trial (Hayes and Jenkins, 1997). Overall repeatability of performance in the four trials was assessed using the intra-class correlation coefficient (I_{cc}) (Lessells and Boag, 1987). To identify whether individuals were consistent performers within the group, for each component of the escape response, individuals were placed in rank-order for each of the four repeat measures and Kendall's coefficient of concordance (W) was used to analyse the consistency of assigned ranks for an individual within the group. In addition, to determine whether individuals that exhibited high or low levels of performance in one escape response measure also exhibited similar levels of performance in other measures, scallops were ranked according to their mean performance for each performance measure and Kendall's coefficient of concordance was used to determine whether individual ranking was consistent (Navas et al., 1999). Sequential Bonferroni corrections were applied to significant results according to the method outlined previously (Holm, 1979) using a family-wise error rate (π) of 0.05.

To determine whether body metrics and calculated condition indices for the queen scallops were size dependent, these measures were regressed against shell size (S_{h2}). Similarly, data for the three condition indices were regressed as the independent variable against the mean of each performance measure to examine whether individual escape performance was related to either somatic condition (MI, CI) or reproductive condition (GSI). Finally, the effect of gonad status on escape performance was examined by regressing maturity stage against the average performance measure for that maturity stage for each of the five escape performance variables measured in this study.

RESULTS
Inter-individual variability in escape response
Significant individual variation was observed in all five measured components of the escape response (Fig. 1, Table 2). Mean response latencies ranged from 1.46 to 4.80 s and the fastest and slowest response latencies observed in any trial were 0.32 and 8.49 s, respectively (Fig. 2). Mean burst clap rates ranged between 2.9 and 4.7 claps s^{-1} and the lowest and highest burst clap rates in any trial were 2.0 and 4.9 claps s^{-1}, respectively (Fig. 2). The lowest and highest average clap rates in any trial were 1.3 and 4.2 claps s^{-1}, respectively (Fig. 1), with mean average clap rates falling between 2.3 and 3.6 claps s^{-1}. Differences between the two clap rate values for an individual scallop ranged from 0.4 to 2.0 claps s^{-1}. The lowest number of claps performed in any trial was 10 whilst the highest
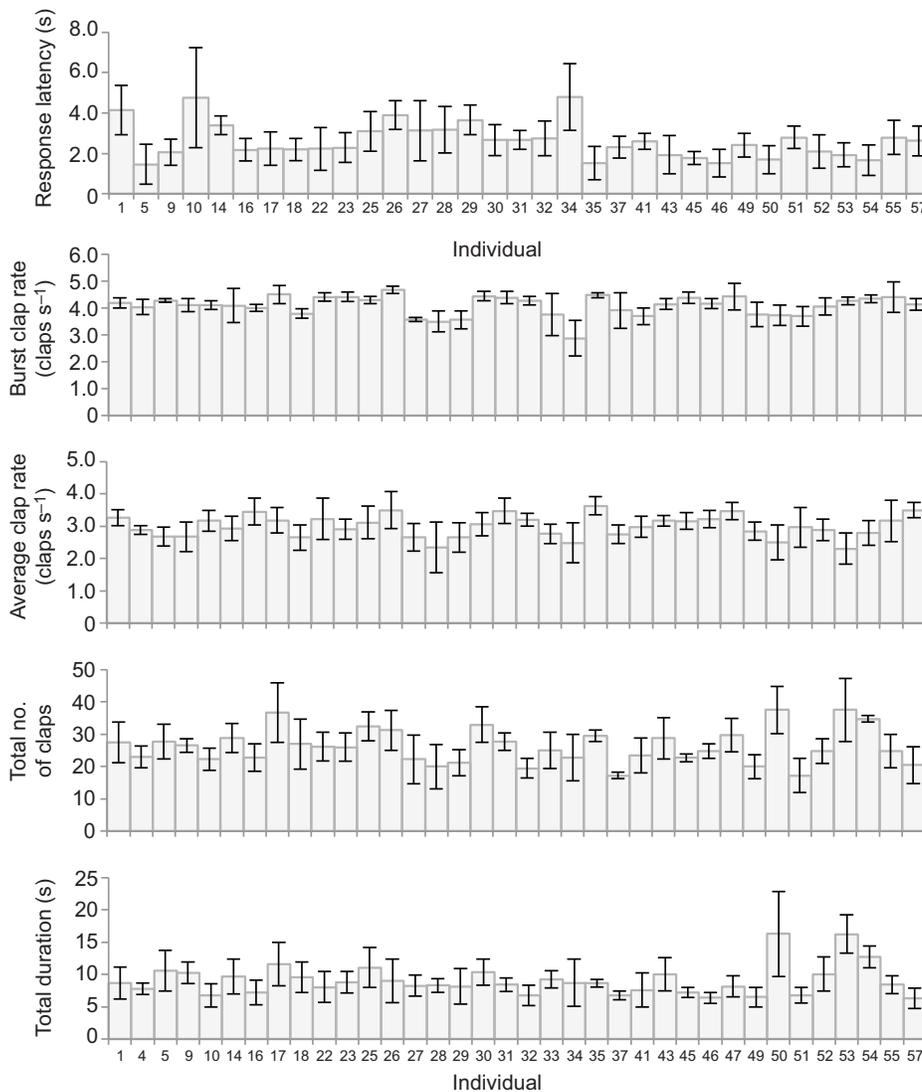

Fig. 1. Inter-individual variability in performance measures of the escape response of the queen scallop (*Aequipecten opercularis*). Data are presented for individuals as means ± s.d. based on four measurements of escape response (days 0, 2, 7 and 28).

Table 2. Repeatability of escape response performance of individual queen scallops (*A. opercularis*)

Performance measure	Test statistic	Repeatability coefficient	P
Response latency (s)	$F_{32,99}=3.02$	$I_{cc}=0.335$	<0.0001
	$\chi_r^2{}_{32,99}=68.69$	$W=0.537$	<0.001
Burst clap rate (claps s^{-1})	–	–	–
	$\chi_r^2{}_{35,108}=88.99$	$W=0.636$	<0.001
Average clap rate (claps s^{-1})	$F_{35,108}=2.71$	$I_{cc}=0.300$	<0.0001
	$\chi_r^2{}_{35,108}=67.26$	$W=0.481$	<0.001
Total number of claps	$F_{35,108}=4.13$	$I_{cc}=0.421$	<0.0001
	$\chi_r^2{}_{35,108}=93.59$	$W=0.672$	<0.001
Total clapping duration (s)	$F_{35,108}=3.50$	$I_{cc}=0.384$	<0.0001
	$\chi_r^2{}_{35,108}=80.63$	$W=0.576$	<0.001

Data are based on four repeat measures (days 0, 2, 7 and 28) using (1) intraclass correlation (I_{cc}) to assess the repeatability of the actual performance measures and (2) Kendall's coefficient of concordance (W) to assess the degree of association across all trials, based on ranked performance of individuals within each trial (corrected for tied ranks).
Note: all data were log$_{10}$ transformed prior to calculation of I_{cc} except for burst clap rate as data remained heteroscedastic after transformation.

was 47 (Fig. 2) with the lowest and highest mean number of claps performed being 17.5 and 37.5 claps, respectively. In 90% of cases, the greatest number of claps was performed in the first phase after stimulation (minimum–maximum, 4–31 claps), with the remaining 10% performing the greatest number in the second phase. Total duration of clapping in any single trial ranged from 4.0 to 26.7 s with the mean total clapping duration ranging between 6.3 and 16.3 s (Fig. 1).

Repeatability of individual escape response performance
Temporal repeatability was significant for all pairwise comparisons of initial *versus* subsequent performance for all performance measures (Fig. 2) except average clap rate at day 0 *versus* day 28, where a trend ($P<0.10$) was found. The general pattern in the data was for temporal repeatability of escape response to decline over time (as indicated by the magnitude of the correlation coefficient) (Fig. 2). Repeatabilities of individual escape responses over the whole trial, based on intra-class correlation analyses, were significant for each performance measure (Table 2; note I_{cc} could not be calculated for burst clap rates). Concordance analyses indicated that individual scallops tended to maintain the same performance ranking within the groups over time for each performance measure (Table 2). Taken together, not only did individual scallops exhibit significant individual repeatability/consistency in all five performance measures over time but also each performance measure ranked in the same order of repeatability/consistency (based on the magnitude of the I_{cc}/W values).

Are scallops high or low performers in all aspects of the escape response?
Individual scallops ranked consistently within the group in terms of mean response latency, burst clap rate, average clap rate and total number of claps (Fig. 3; $\chi_r^2{}_{32,132}=60.84$, $W=0.475$, $P<0.005$). (Note: total clapping duration was excluded from the analyses on the grounds that it might exaggerate the degree of consistency/significance artificially, being dependent, in part, upon the total number of claps.) Thus, individuals who ranked highly in one measure tended to rank highly in all and displayed some of the shortest response latencies, highest clap rates and greatest total
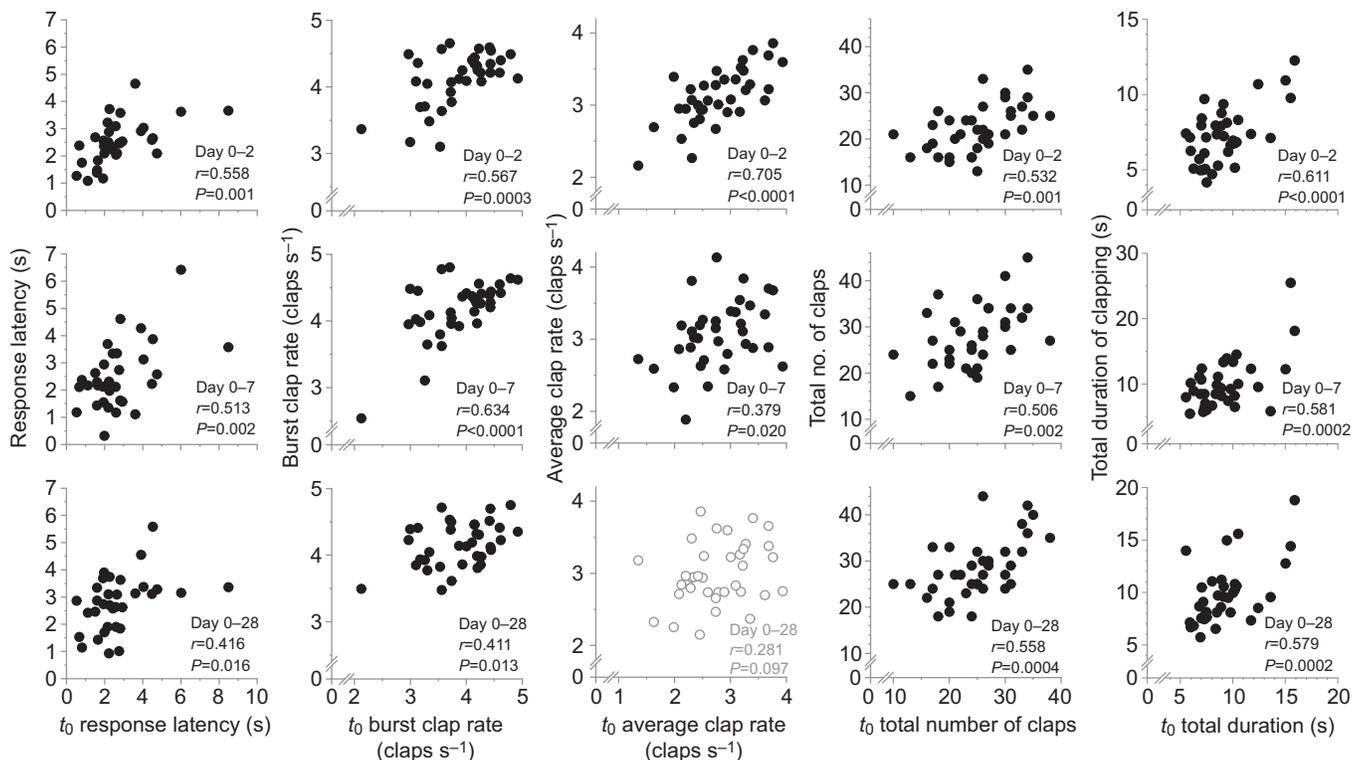

Fig. 2. Repeatability of performance measures of the escape response of *A. opercularis* measured on four occasions (days 0, 2, 7 and 28). Scatterplots present covariance patterns between initial performance on day 0 and subsequent performance on days 2 (upper), 7 (middle) and 28 (lower) for each measure. Significant repeatabilities (Pearson's product-moment correlation) are plotted as solid black points and non-significant repeatabilities as open grey points.
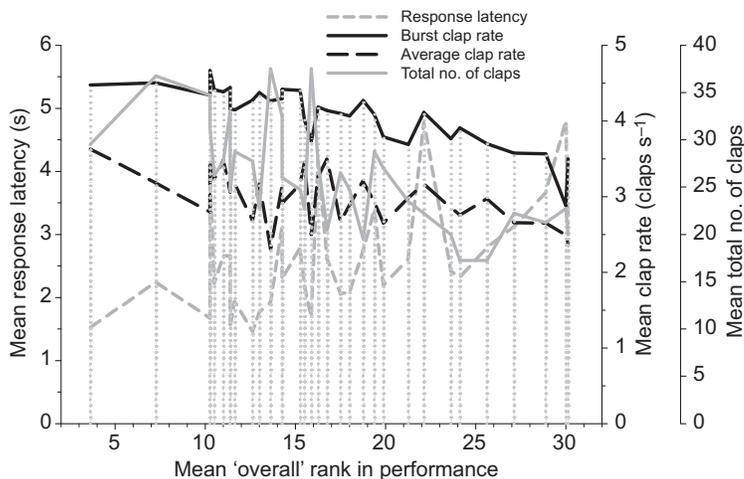
Fig. 3. Individual mean rank in escape response performance for *A. opercularis*. Rankings for each scallop (*N*=33, indicated by dotted, vertical lines on plot; includes three tied rank pairs) are based on their ranking within the group for mean response latency (shortest–longest), mean burst and average clap rate (fastest–slowest) and mean total number of claps (largest–smallest) for the four measurement days. The mean 'overall' rank in performance (*x*-axis) was calculated as the overall mean for these four performance rankings and ranged from 3.6 for the best performer to 30.1 for the poorest performer.

number of claps compared with those with the poorest overall ranking (Fig. 3). Amongst the 33 scallops included in this analysis, there were two consistently high and four consistently low performers (Fig. 3). The distribution of the mid-ranking scallops was more condensed indicating greater rank switching between these individuals and more variable levels of performance in response latency and total number of claps (Fig. 3).

Is individual escape performance related to maturity status or condition?

Body metrics and calculated condition indices are presented in Table 1. GSI displayed large variability across individuals and within most gonad maturity stages (Table 1) and was independent of size (regression *versus* S_{h2}, $F_{1.39}$=0.46, P=0.53). BI and MI were also variable between individuals, exhibiting, respectively, a 3-fold and 2-fold variation in magnitude (Table 1) but were independent of size (regression *versus* S_{h2}; CI, $F_{1.39}$=0.13, P=0.91; MI, $F_{1.39}$=0.18, P=0.68). To examine whether individual escape performance was related to condition, the mean of each performance measure was regressed against somatic condition (MI, CI) or reproductive condition (GSI). Latency and clap rates did not show any relationship to the condition indices (all P>0.05). However, the endurance components of the escape response (total number of claps, clapping duration) were linearly related to somatic condition with an increase in MI or CI resulting in increased endurance in the escape response (see supplementary material Table S1). Visual inspection of the size and appearance of the gonads indicated that 83% of individuals were graded 3–5 (pre-spawning stages) with 12% classed as immature and 5% as 'spent' (Table 1). No aspect of escape response performance was related to maturity stage.

DISCUSSION

This study found significant inter-individual variability in each measure of escape response performance and significant repeatability in performance over the 28 days. However, it is important to note that the range of individual escape responses recorded in this study is for wild scallops that have survived to a size of 60–70 mm shell height (likely age 3–5 years) (see Philipp et al., 2006). Given the heavy predation pressure on juvenile scallops (Barbeau et al., 1996; Kamenos et al., 2004), it is likely that poorer performing phenotypes have already been removed from this cohort. In addition, some high performing individuals may also have avoided capture by the dredge (Jenkins and Brand, 2001). Therefore, the full range of escape response performance originally present in this

cohort will not have been recorded. Nevertheless, this study has shown the considerable inter-individual variation in physiological performance upon which natural selection can operate through selective predation mortality.

Response latencies in any given trial ranged from 0.32 and 8.49 s, exhibiting a 30-fold variation between individuals. Whether these latencies have significant consequences for survival will depend upon the minimum time required for a predator to gain a sufficient hold upon the scallop for predation to be successful and on the size, strength, speed and experience of the predator in question (Ordzie and Garafalo, 1980) but response latency may be closely linked to scallop fitness (Brokordt et al., 2012). Given that scallops only initiated escape responses upon direct contact of the sensory mantle tentacles with the predator (allowing unchecked shell contact prior to this), there is clearly considerable scope for starfish to successfully select against greater latency performers. Similarly, clapping performance varied between individuals with some scallops producing, on average, twice as many claps or clapping for twice as long as others. The advantage gained from superior locomotor endurance (inferred as a function of the duration spent clapping and/or the total number of claps) may be in the evasion of persistent mobile predators (Barbeau and Scheibling, 1994) and could also increase the likelihood of survival for an individual during multiple predator encounters in a short time period, for example for juvenile queen scallop on mäerl bed nursery grounds (Kamenos et al., 2004). It is interesting to note that greater inter-individual variability was found in average clap rate compared with burst clap rate, which suggests that some individuals are capable of sustaining a superior level of performance during their escape response (assuming that clap rate correlates with increased force production and a greater distance travelled during the escape response).

It is important to note that although we measured a number of facets of the escape response of a scallop, we did not measure force production as has been recorded for other scallop species. In addition, because of restraint during stimulation, swimming speed or distance travelled during the escape response was not measured and so the translation of the variable individual clap rates into an actual three-dimensional spatial response was not possible in this study. Force production measured during the escape responses of *Placopecten magellanicus* (Fleury et al., 2005; Guderley et al., 2008; Guderley et al., 2009) and *Argopecten purpuratus* (Pérez et al., 2009; Guderley et al., 2011) has shown considerable inter-individual variation. Similarly, data presented for the relationship between number of claps and the linear distance travelled by *Pecten maximus*

indicates considerable variation in the distance travelled by individuals for a given number of valve adductions (Jenkins and Brand, 2001). Therefore, it is possible that some scallops recording lower clap rates in our study may be producing greater average force output during valve adduction, resulting in an equivalent or greater escape response in terms of swimming speed and distance travelled to that of individuals performing faster clap rates. Clearly future work should examine the relationship between clap rate, force production and distance travelled.

In this study, individual escape response performance was not related to maturity stage although some aspects of the escape response, i.e. total number of claps and clapping duration (the endurance components) were linearly related to muscle and body condition index. Previous studies have shown that the process of maturation has no effect on escape responses in most scallop species (Brokordt et al., 2000a; Brokordt et al., 2000b; Pérez et al., 2009; but see Kraffe et al., 2008) although recuperation rates following exhaustive exercise are affected by maturity stage, with mature and spawned scallops taking longer to recover. However, as complete recovery from exhaustive exercise within 18 h has been shown for all reproductive stages in pectinids (Brokordt et al., 2000a; Brokordt et al., 2000b; Kraffe et al., 2008; Pérez et al., 2009) and the minimum time interval between repeat measures in this study was 48 h, all scallops in this study were fully recovered from exhaustive exercise between each assessment of escape response performance and so maturity stage will not have had any effect on escape response performance.

To our knowledge, the repeatability of locomotor performance in invertebrates has only been quantified in three studies: on ants (Clusella-Trullas et al., 2010), spiders (Prenter et al., 2010) and the scallop *A. purpuratus* (Brokordt et al., 2012). Brokordt and colleagues (Brokordt et al., 2012) recently examined the repeatability of the response latency, total number of claps and clapping duration for escape responses conducted 5 min apart and obtained significant repeatabilities of 0.40, 0.36 and 0.42, respectively. If the magnitude of the correlation coefficient is taken as a measure of the degree of repeatability (1.00=perfect repeatability), then these values represent moderate levels of repeatability and are lower than the repeatabilities obtained over longer time intervals in the present study. Physiological changes within the adductor muscle following the first bout of intense physical activity (reviewed in Brokordt et al., 2012) and very little recovery time can explain the reduced performance recorded in the second performance trials 5 min later. However, the correlated performance levels in the two trials show that high performing individuals would be more likely to escape two predation attempts occurring in quick succession as might be the case where predators are abundant (Barbeau et al., 1996) or persistent in pursuing their prey (Barbeau and Scheibling, 1994). In contrast, the time intervals between repeat measures in our study allowed complete recovery from exhaustive exercise (Bailey et al., 2003) and provide measures of the repeatability of maximum escape response performance over time.

In the present study, the general pattern observed was for repeatability to decline over time in the sensory acuity (i.e. response latency) and swimming performance (i.e. burst and average clap rates) components of the escape response whilst the level of repeatability for swimming endurance (i.e. total number of claps and clapping duration) was maintained over the 28 day experiment. This tendency for repeatability to decrease with increasing time interval between repeat measures has been observed in previous studies on locomotor performance (e.g. van Berkum et al., 1989; Austin and Schaffer, 1992; but see Elnitsky and Claussen, 2006).

Published data for the repeatability of burst locomotor performance collated from studies on Arthropoda, Amphibia, Osteichthyes and Reptilia, in addition to the scallop data from the present study are summarised in supplementary material Table S2 and presented in Fig. 4. The repeatability measures obtained for burst clap rate in this study are within the range of repeatability values expected for the given time interval between repeat measures (i.e. within the range of 0.5–0.7; Fig. 4). Our meta-analysis indicates that repeatability coefficients for burst locomotor performance tend to be very variable in the very short term (i.e. seconds to hours) ranging between 0.18 and 0.92 (mean 0.67±0.22). In contrast, repeatability tends to be higher for time intervals between repeat measures of 1 day and 2 weeks, with repeatability coefficients of 0.43–0.95 (mean 0.64±0.18). A variable and reduced level of repeatability in the very short term is to be expected as animals may not be fully recovered from exhaustive or sustained exercise between the repeat measures (Gleeson, 1991; Milligan, 1996; Bailey et al., 2003). A further increase in time interval of the order of months results in a decline in repeatability with values of 0.18–0.76 (mean 0.41±0.14). This decline in repeatability over time is most probably due to the effects of senescence on locomotor performance (e.g. Reznick et al., 2004; Bronikowski et al., 2006) or decoupling of performance following metamorphosis in amphibians (Johansson et al., 2010). In scallops, changes in both muscle physiology and escape response performance with age and size have been observed (Tremblay et al., 2006; Phillipp et al., 2008; Schmidt et al., 2008; Lebrecque and Guderley, 2011).

Finally, in this study we determined whether individual scallops were consistently high or poor performers in all aspects of the escape response. Recently, Brokordt and colleagues (Brokordt et al., 2012) have shown positive pairwise correlations between different aspects of the escape response of juvenile *A. purpuratus*. Individuals that responded more quickly also tended to exhibit a longer clap duration and produce a greater number of claps. Using concordance analysis our results indicated that out of the 33 scallops ranked across four escape performance measures, there were two consistently high and four consistently low performers with more variable levels of performance and ranking for mid-ranking individuals. Navas and colleagues (Navas et al., 1999) examined the locomotor performance, in terms of jumping and swimming, of the frog *Rana temporaria* to determine whether some frogs were better athletes by having consistently higher locomotor performance in both terrestrial and aquatic environments. The results indicated that the poorest performers exhibited performance levels that were 40%
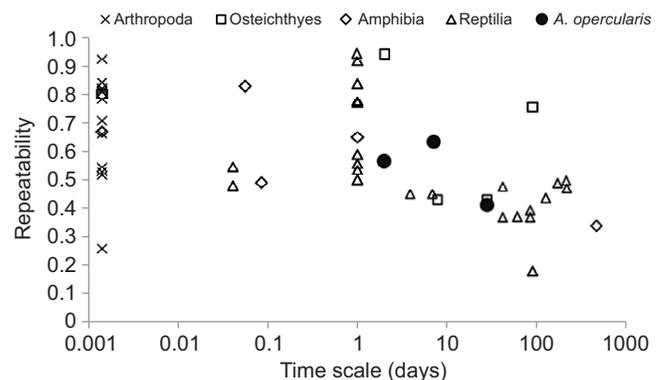

Fig. 4. The effect of increasing time interval between repeat measures on the repeatability of burst locomotor performance in various taxa (supplementary material Table S2). Values presented for *A. opercularis* from this study are for burst clap rate.

lower than the highest performers and that some frogs had an overall greater capacity for locomotor performance in both environments (although actual performance was strongly influenced by temperature). In our study, the poorest performing scallops exhibited performance levels that were 22–33% lower than those of the high performers for endurance (total number of claps) and maximum and sustained performance (burst and average clap rate) measures, and response latencies (a measure of sensory acuity) that were 167% higher.

In conclusion, our study has demonstrated significant repeatability in the escape responses of the queen scallop, *A. opercularis*, when presented with a predatory threat from the starfish *A. rubens*. In addition, our analyses indicate that individual scallops exhibit consistent levels of performance ranking, with some individuals performing as consistently high or low performers across all aspects of the escape response measured. A scallop's ability to evade predators through the provision of an escape response of an appropriate magnitude, subject to physiological, behavioural and organismal constraints, will have clear fitness-related consequences (Bennett, 1987; Bennett and Huey, 1990; Brokordt et al., 2012).

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the study and manuscript. I.D.M. conceived the study; I.D.M, S.R.L. and S.R.J. designed the study; S.R.L. carried out the experiments; S.R.L. analysed the data and prepared the figures; S.R.L., I.D.M. and S.R.J. interpreted the findings; I.D.M. and S.R.L. wrote the draft of the paper and S.R.J. provided comments/input to the manuscript at the draft stage.

COMPETING INTERESTS

No competing interests declared.

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