## INTRODUCTION

Locomotor ability is a key organismal performance trait in the chain of interactions that link biochemical, morphological and physiological traits to fitness (Arnold, 1983). For many species, essential ecological functions such as predator–prey interactions, reproductive activities or foraging, are dependent upon an animal's capacity for movement. The intuitive recognition of this relationship has led to a consistent interest in fish locomotion among scientists, but the nature of the relationship between locomotion and fitness remains largely unknown for most fish species.

The study of fish locomotion began in earnest around 60 years ago (e.g. Black, 1955; Bainbridge, 1958a; Bainbridge, 1958b; Brett, 1964; Fry, 1971; Beamish, 1978) but there are still large gaps in our understanding of the elements that determine swimming performance. For instance, the nature of maximal swimming performance as well as the factors which contribute to the transition between aerobic and anaerobic swimming modes, and to exhaustion, are still poorly understood. Furthermore, we know almost nothing about the ecological and evolutionary relevance of aerobic or anaerobic swimming abilities (e.g. Lankford et al., 2001) and whether natural selection operates on them. For instance, it is unknown whether aerobic and anaerobic performance are linked or whether they follow independent evolutionary trajectories. Quite clearly, investigating how individual fish from wild populations respond to locomotor challenges is one way to explore the link between swimming performance and fitness (Kolok, 1999; Plaut, 2001; Nelson and Claireaux, 2005).

To investigate whether swimming performance responds to natural selection, three different issues must be considered: (1) the performance trait must show variation amongst individuals that is relatively stable over time – i.e. theirmance ast bst e a2si

proven to be repeatable, even across thermal regimes, surgical treatments and 6 months of mesocosm residence (Kolok, 1992; Butler et al., 1989; Kolok and Farrell, 1994; Claireaux et al., 2007). Sprint swimming, which is used to capture prey or avoid predatory pursuit, also exhibits much intraspecific variation that is relatively stable, being repeatable over time periods spanning from hours to months (Reidy et al., 2000; Nelson et al., 2002; Nelson and Claireaux, 2005; Nelson et al., 2008) and across different thermal and nutritional conditions (Martinez et al., 2002; Claireaux et al., 2007). There is also evidence that fast-start performance, which is a critical component of some 'sit and wait' predator–prey encounters, is stable and repeatable over hours and weeks (Fuiman and Cowan, 2003) (S.M., unpublished observations). Constant acceleration tests (CATs), the focus of the present study, have been shown to be repeatable in the Atlantic cod (Reidy et al., 2000). This type of test evaluates a swimming performance that fish may employ when manoeuvring through strong currents, being pursued by strong swimming predators, or trying to escape a fishing trawl.

The European sea bass *Dicentrarchus labrax* L. is a temperate perciform species that is economically important in the Mediterranean and western Atlantic. They are active predators, which catch their prey by pursuit, and adults are known to swim over 1000 km to forage and reach spawning grounds (Pickett and Pawson, 1994). The species has a complex life cycle; spawning occurs offshore in late winter and the pelagic larvae hatch in the open sea. They drift inshore and colonize sheltered transitional coastal habitats in the spring, in particular lagoons and estuaries, where they metamorphose to juveniles and grow for their first summer (Pickett and Pawson, 1994; Dufour et al., 2009). Predation pressure by other fish and birds may cause a significant number of mortalities during this phase (Quignard et al., 1984; Dufour et al., 2009). The sea bass leave the lagoons and estuaries as temperatures drop in autumn, but they continue facultative seasonal migrations between the open sea and the transitional habitats as they grow to maturity. Thus, European sea bass have a life cycle that intimates an important role for locomotion, in which both anaerobic burst swimming and sustained aerobic swimming performance could potentially influence an individual's ability to survive, grow to maturity and reproduce.

In the present study, individual variation in a CAT, and its repeatability over the short term (minutes), was investigated in European sea bass. The CAT can be completed much more rapidly than the more widely used $U_{crit}$ protocol [minutes *vs* hours (Nelson et al., 2002; Farrell, 2008)] and can provide a measure of both aerobic and anaerobic performance. Sea bass performance was measured consecutively four times with a 5 min interval between each CAT. The fish were filmed to analyse changes in 1tdua66.4999f: aerobic

over 2 h (measuring period: 10 min; flushing period: 10 min). After at least five of these $\dot{M}_{O2}$ routine measurements, a CAT was performed as described previously (Reidy et al., 2000). Briefly, water velocity was increased steadily by $10 \, \text{cm s}^{-1} \, \text{min}^{-1}$ until exhaustion. Exhaustion was defined as the fish falling back against the retaining grid at the posterior end of the swim tunnel and being unable to resume swimming. Water velocity at exhaustion was considered to be the maximum speed attainable by the fish using its highest power output, assuming that ground speed was essentially zero, and was recorded as $U_{CAT}$. At $U_{CAT}$, water velocity was immediately decreased to the initial velocity of $30 \, \text{cm s}^{-1}$ and oxygen uptake monitored for the following 5 min, after which the whole procedure was repeated three more times. At the end of the final trial, water velocity was returned to $30 \, \text{cm s}^{-1}$ and $\dot{M}_{O2}$ measured for at least 4 h (measuring time: 12 min; flushing time: 12 min). The highest $\dot{M}_{O2}$, measured throughout the 5 min of rest immediately following each CAT, was designated maximum $\dot{M}_{O2}$ (MMR). MMR was then used to calculate an available aerobic scope for each fish by subtracting the RMR. Individual recovery rate was assessed as the time required for oxygen uptake to return from the maximal post-exercise $\dot{M}_{O2}$ to 50% of RMR. Fish were then removed from the swim tunnel, anaesthetized (2-phenoxyethanol; $0.3 \, \text{ml l}^{-1}$) and measured for length, width, depth and mass. Finally, background oxygen consumption was assessed by monitoring oxygen consumption of the respirometer without any fish, at a current speed of $30 \, \text{cm s}^{-1}$ for 1 h.

### Data analysis and statistics

Values are means ± s.e.m. and significance was accepted at the $P<0.05$ level. Statistical analyses were performed using SigmaStat 3.1 (Systat software; www.systat.com) and Statistica 5.0 (Statsoft; www.statsoft.com). A statistical comparison between the four swim trials was carried out using a parametric analysis of variance for repeated measures (rm ANOVA) and a *post hoc* Tukey test was used to identify differences between trials. Repeatability was tested by two different procedures. The first procedure used the Spearman rank order test to investigate repeatability of the swimming performance for individual fish in successive trials, and a Kendall concordance coefficient (KCC) was calculated to assess repeatability across multiple trials. The second procedure used the intraclass correlation coefficient (ICC). This coefficient is the ratio of variance among individuals to the total variance (among+within) and is calculated from the mean square terms of the ANOVA (Becker, 1984; Lessells and Boag, 1987). The ICC ranges from 0 to 1. It is 0 when all individuals have the same mean, while it is 1 when all individuals have a different mean and all measurements on the same individual are identical (i.e. perfect repeatability). Pearson correlation was used to detect any relationship between recovery time and other variables. A probability of less than 5% ($P<0.05$) was taken as the limit for statistical significance.

### RESULTS
### Constant acceleration performance

Although fish ranged from 124 g to 297 g in mass, and from 22.7 cm to 31 cm in fork length, size did not significantly influence CAT swimming performance ($U_{CAT,mass}$, $P$=0.40; $U_{gt,mass}$, $P$=0.71; $U_{CAT,length}$

throughout the test up to $U_{gt}$. Beyond gait transition, during the bursts of propulsive thrust, TBA was significantly wider than during the aerobic swimming (ANOVA, $P$<0.01; Fig. 3). Once initiated, the mean number of burst thrusts increased steadily as water velocity increased (Fig. 4).

### Post-exercise metabolism and recovery from exhaustive swimming

Inter-individual variation in metabolic rate exceeded intra-individual variation in replicate measurements for all periods of the experiment. For the period prior to the CAT (swimming at $30\,\mathrm{cm\,s^{-1}}$), the RMR varied among individuals from 7.56 to 30.28 m

Overall, sea bass performed significantly better in the first CAT, because all but two of the 16 individuals had their best performance during the first test. Subsequent tests were remarkably homogeneous both when analysed as group mean and when analysed as individuals. Pedersen and Malte (Pedersen and Malte, 2004) subjected brown trout (*Salmo trutta*) to multiple CATs over a short time period (30 s recovery time between consecutive trials) but did not report the actual individual $U_{CAT}$

In contrast to the inferred anaerobic support of performance, the presumed limit of aerobic-only exercise ($U_{gt}$) was much less variable across all four tests, although performance improved significantly from CAT 1 to 4. The increase in $U_{gt}$ may be a consequence of several phenomena associated with exhaustion induced by CAT 1, among which is the release of catecholamines into the bloodstream (Tang et al., 1989). Such a release has been shown to improve oxygen transport (Claireaux et al., 1988), to support oxygen delivery to the tissues (Tang et al., 1989) and to facilitate the mobilization of energy stores (Gamperl et al., 1994). The potential physiological and/or behavioural mechanisms underlying this improvement in aerobic performance after the first CAT will require further study. Nonetheless, the intra-individual repeatability of aerobic metabolism and $U_{gt}$ coupled with relatively small inter-individual variation in these variables signals the importance of preserving maximal aerobic function at all times in this species and may indicate that these traits are subject to stabilizing selection.

### Post-exercise metabolism and recovery from exhaustive swimming

As has been reported previously, metabolic rate was highly variable among individual sea bass (Claireaux and Lagardere, 1999; Nelson and Claireaux, 2005; Claireaux et al., 2007), a finding in common with those in other fish species (Farmer and Beamish, 1969; Febry and Lutz, 1987; Tang et al., 1994). Some authors have attributed this high degree of variation to confinement or handling stress, and to measurement errors (Febry and Lutz, 1987; Steffensen, 1989). However, the individual repeatability of metabolic rate while swimming has received very little attention. In the present study, the rank order of oxygen consumption among 16 sea bass was significantly repeatable across all phases of the experiment, i.e. under routine conditions (swimming at $30\,\mathrm{cm\,s^{-1}}$), immediately following exhaustion and over the final 3.5h recovery period after the four consecutive CAT tests. While this unprecedented level of repeatability in ranking indicates that variation in metabolic rate may be of ecological significance and a potential determinant of fitness in sea bass, future research should confirm whether the variation is stable over longer time periods.

The measurement of $\dot{M}_{O_2}$ after the four consecutive CATs revealed two groups of animals that differed in their pattern of recovery. Animals that recovered relatively quickly, within about 40min, tended to have higher metabolic rates during routine swimming ($30\,\mathrm{cm\,s^{-1}}$) and a higher aerobic scope, but were unable to reach as high a $U_{CAT}$ as the other group of animals that relied more upon anaerobic support for swimming. Those animals that achieved the highest $U_{CAT}$, with the most extensive use of the burst-and-coast swimming mode, did so at the expense of an approximately fivefold increase in their recovery period compared with the animals that used less anaerobic support. This indicates the possibility of a trade-off between speed of recovery and maximal swimming performance. Moreover, the fact that the rate of recovery was significantly related to RMR and aerobic scope suggests that expensive morphological traits that facilitate high rates of aerobic metabolism, such as a high density of mitochondria, large hearts and higher capillary densities (etc.), may be the outcome of natural selection for fast recovery from exercise and not for exercise performance *per se*. Although the physiological basis of this apparent trade-off remains to be determined, the presence of two groups of fish, one composed of cruising specialists (higher aerobic performance, quick recovery from strenuous swimming, but low maximum burst speeds) and a second group of sprinting specialists (higher maximum burst speeds, but lower aerobic performance and slow recovery from strenuous swimming), may be the result of disruptive (or diversifying) selection. There may be advantages for cruising specialists in long-distance foraging and seasonal migration whereas sprinting specialists may be favoured in predator avoidance and prey capture.

### LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| CAT | constant acceleration test |
| CV | coefficient of variation |
| EPOC | excess post-exercise oxygen consumption |
| ICC | intraclass correlation coefficient |
| KCC | Kendall concordance coefficient |
| MMR | maximum metabolic rate |
| $\dot{M}_{O_2}$ | oxygen consumption |
| PCr | phosphocreatine |
| RMR | routine metabolic rate |
| TBA | tail beat amplitude |
| TBF | tail beat frequency |
| $U_{CAT}$ | |

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