The Journal of Experimental Biology 214, 1821-1828

doi:10.1242/jeb.052753

RESEARCH ARTICLE

Post-prandial changes in protein synthesis in red drum (*Sciaenops ocellatus*) larvae

Ian D. McCarthy[1,*] and Lee A. Fuiman[2]

[1]School of Ocean Sciences, College of Natural Sciences, Bangor University, Menai Bridge, Anglesey LL59 5AB, UK and [2]Marine Science Institute, The University of Texas at Austin, 750 Channel View Drive, Port Aransas, TX 78373, USA

*Author for correspondence (i.mccarthy@bangor.ac.uk)

Accepted 22 February 2011

SUMMARY

Protein synthesis is one of the major energy-consuming processes in all living organisms. Post-prandial changes in protein synthesis have been studied in a range of animal taxa but have been little studied in fish larvae. Using the flooding-dose method, we measured post-prandial changes in whole-body rates of protein synthesis in regularly fed red drum *Sciaenops ocellatus* (Linnaeus) larvae for 24–28 h following their daily meal. Fractional rates of protein synthesis increased from a baseline (pre-feeding) rate of 16% day^{-1} to a post-prandial peak of 48% day^{-1} ca. 8 h after feeding before declining to 12% day^{-1} after 24–28 h. The overall mean daily rate of protein synthesis was calculated as 27% day^{-1}. Although suggested as energetically impossible in larval poikilotherms, our results show that rates in excess of 30% day^{-1} can be attained by larval fishes for a few hours but are not sustained. The average daily energetic cost of protein synthesis was estimated as 34% of daily total oxygen consumption, ranging from 19% immediately before feeding to 61% during the post-prandial peak in protein synthesis. This suggests that during the post-prandial peak, protein synthesis will require a large proportion of the hourly energy production, which, given the limited metabolic scope in fish larvae, may limit the energy that could otherwise be allocated to other energy-costly functions, such as foraging and escape responses.

Key words: *Sciaenops ocellatus*, protein synthesis, post-prandial metabolism, energetics, fish larvae.

INTRODUCTION

One of the major energy-consuming processes in all living organisms is the synthesis of protein to be used for maintenance (i.e. to replace existing protein) or for elaboration of new tissue (growth, reproduction). For example, protein synthesis can account for 20% of basal energy expenditure in man (Reeds et al., 1985) and between 11 and 49% of total energy expenditure in fishes (Houlihan et al., 1995a; Carter and Houlihan, 2001). Protein breakdown, part of the continual cycle of protein turnover whereby existing organismal proteins are broken down and replaced by newly synthesised proteins (Houlihan et al., 1995a), accounts for a further energetic cost. Although this cost has not yet been fully quantified in fishes, it may account for as much as 19% of total energy expenditure in mammals (Fraser and Rogers, 2007). Because of its central role in energetics and growth, animal physiologists have shown much interest in the study of protein metabolism (i.e. the cycle of protein synthesis, breakdown and accretion) (Waterlow, 2006), and in fishes the effects of various abiotic (e.g. temperature, salinity and oxygen levels) and biotic (e.g. nutritional status, size and tissue and/or organ type) factors on these processes have been summarised in several reviews over the last 20 years (Houlihan, 1991; Houlihan et al., 1995a; Houlihan et al., 1995b; Carter and Houlihan, 2001; Fraser and Rogers, 2007).

The larval period in fishes has been recognised as a crucial stage in the life cycle, characterised by two energy-costly processes, both of which rely on protein synthesis: ontogeny (rapid differentiation of new tissues and organs) and rapid growth (increase in body size) (Fuiman, 2002). Fish larvae face considerable challenges in maintaining energy balance (Wieser, 1995; Pedersen, 1997), yet data on rates of protein synthesis in fish larvae are much more scarce than for juvenile or adult fishes because of methodological problems of adapting existing protein synthesis techniques to work with fish larvae (Houlihan et al., 1995c; Fraser and Rogers, 2007). Most studies of fish larvae have focused on relating rates of protein synthesis to growth and determining the energetic costs of protein synthesis (Houlihan et al., 1992; Houlihan et al., 1993; Houlihan et al., 1995d; Conceição et al., 1997a; Smith and Ottema, 2006), although the effects of temperature (Mathers et al., 1993) and contaminant exposure (McCarthy and Fuiman, 2008) have been reported.

Following a meal, a series of physiological changes occurs in animals as they digest, absorb and assimilate ingested nutrients (Carter et al., 2001). The post-prandial increase in metabolic rate, known as specific dynamic action (SDA), is the most studied physiological response to ingestion of nutrients as it provides an integrated measure of the energy expended on all activities of the body involved in the processing of a meal (McCue, 2006; Secor, 2009). Because protein synthesis represents the major energetic cost of processing a meal, post-prandial changes in protein synthesis have been studied in a range of animal taxa: crustaceans (e.g. Houlihan et al., 1990; Whiteley et al., 2001), fishes (e.g. McMillan and Houlihan, 1988; Lyndon et al., 1992; Katersky and Carter, 2010), birds (e.g. Yaman et al., 2000), mammals (e.g. Simon and Bergner, 1983; Yoshizawa et al., 1995; Danicke et al., 1999) and humans (e.g. McNurlan et al., 1993; Welle et al., 1994; Waterlow, 2006). For juvenile and adult fishes, there are tissue-specific and temperature-dependent responses in the timing and magnitude of the post-prandial peak in protein synthesis (McMillan and Houlihan, 1988; Fauconneau et al., 1989; McMillan and Houlihan, 1989; Lyndon et al., 1992; Negatu and Meier, 1993; Katersky and Carter,

2010). In contrast, knowledge of post-prandial changes in protein synthesis in fish larvae is limited to a single study (Houlihan et al., 1993).

One feature common to all studies of the post-prandial response of protein synthesis in fishes is limited sampling over time following a meal. Most studies have sampled at 2 to 12 h intervals over a 12–24 h period after feeding (McMillan and Houlihan, 1988; McMillan and Houlihan, 1989; Fauconneau et al., 1989; Lyndon et al., 1992; Katersky and Carter, 2010). This can result in either an incomplete time course when the response extends beyond the sampled period (e.g. McMillan and Houlihan, 1988; McMillan and Houlihan, 1989; Fauconneau et al., 1989) or a coarse temporal resolution as a result of large time intervals between samples (e.g. Lyndon et al., 1992; Katersky and Carter, 2010). Conclusions about the timing and magnitude of post-prandial changes in protein synthesis may be improved by more regular sampling over time following a meal. In addition, many studies of post-prandial changes in protein synthesis have starved fish for 6–7 days prior to refeeding and measuring rates of protein synthesis (McMillan and Houlihan, 1988; McMillan and Houlihan, 1989; Fauconneau et al., 1989; Lyndon et al., 1992) compared with regularly fed fishes (Houlihan et al., 1993; Katersky and Carter, 2010). Given that rates of protein synthesis and breakdown are known to be influenced by feeding/starvation history (Carter and Houlihan, 2001; Fraser and Rogers, 2007), it is possible that these factors may also affect the timing and magnitude of post-prandial changes in protein synthesis.

The aim of this study was to measure post-prandial changes in whole-body rates of protein synthesis in regularly fed red drum *Sciaenops ocellatus* (Linnaeus) larvae. We chose red drum as an experimental animal as it is a fast growing marine fish that is an excellent model for the study of physiological energetics in larval fishes (Rooker and Holt, 1996; Torres et al., 1996; Herzka and Holt, 2000; McCarthy and Fuiman, 2008). We measured rates of protein synthesis at regular intervals over a 28 h period after a meal in order to describe the post-prandial response in detail.

MATERIALS AND METHODS

Larval rearing and feeding

Red drum larvae were reared at the University of Texas Marine Science Institute (Port Aransas, TX, USA). Eggs were obtained from broodstock (multiple males and females per tank) induced to spawn by temperature and photoperiod manipulations (Arnold, 1988) at the Marine Science Institute's Fisheries and Mariculture Laboratory (FAML). Three spawns (replicate trials) were used in the experiment. Eggs were collected within 12 h of spawning and transported to the laboratory where they were cleaned in a 0.1% formalin solution (Fisher Scientific International, Hampton, NH, USA) for 30 min, rinsed and placed in 20 litres of sea water mixed with green algae (*Nannochloropsis oculata*) in 150-litre conical rearing tanks at a density of 150–200 eggs l^{-1}. Hatching occurred overnight. Beginning 6 days post-hatching, the volume of water in each tank was gradually increased over the next 5 days until it reached 100 litres, after which a filter was added and recirculating flow was begun. Rearing temperature and salinity were 27.4±0.2°C (mean ± s.d.) and 27.7±0.2 PSU (practical salinity units), respectively. Beginning on day 2 post hatching, rotifers (*Brachionus plicatilis*) that had been enriched with algae (*Isochrysis galbana*) for 2 h, were added to rearing tanks at a rate of 10 rotifers ml^{-1} day^{-1}. From days 10 to 14 post hatching, rotifers were gradually replaced in the diet with brine shrimp (*Artemia salina*) nauplii. Brine shrimp were enriched with Algamac 2000 (Aquafauna Bio-Marine Inc., Los Angeles, CA, USA) for 24 h and added to fish tanks at a rate of 5 nauplii ml^{-1} day^{-1}. Larvae were fed once daily between 09:00 and 11:00 h on a 12h:12 h light:dark photoperiod. The tanks were siphoned daily to remove debris from the bottom. Visual observation indicated that most *Artemia*, which collected at the water surface, were eaten within this 2 h feeding period. Larvae reached the target size for experimentation, i.e. settlement size of 7–9 mm total length (60–68% developed) (Fuiman et al., 1998), within 17–21 days post-hatching. Once larvae in the three replicate spawns reached settlement size, groups of larvae were removed from the tanks to measure rates of protein synthesis. The mean (±s.d.) total lengths (TL) of the larvae from the three replicate trials were 6.4±0.4, 7.7±0.3 and 6.8±0.3 mm, respectively. The corresponding mean (±s.d.) wet masses of the larvae were 3.35±0.58, 5.62±0.61 and 3.91±0.44 mg.

Protein synthesis

Protein synthesis measurements were made using the flooding-dose method of Garlick et al. as modified for larval fishes by Houlihan et al. and applied to red drum larvae by McCarthy and Fuiman (Garlick et al., 1980; Houlihan et al., 1995a; McCarthy and Fuiman, 2008). A solution of 135 mmol l^{-1} phenylalanine (Phe; pH 8) containing L-[2,6-^{3}H]Phe (3.7 MBq ml^{-1}; Amersham Pharmacia, Piscataway, NJ, USA) was diluted 1:100 with sea water to produce a bathing solution with a measured specific radioactivity of 1087±137 d.p.m. $nmol^{-1}$ Phe. Rates of protein synthesis were measured in groups of larvae (15–30, depending on larval size; mean=24 larvae) with an approximate total wet mass of 100 mg [see McCarthy and Fuiman (McCarthy and Fuiman, 2008) for more details on how sample sizes were determined]. Larvae were placed in 100-ml incubation cups filled with 25 ml of bathing solution with light aeration and left for fixed periods of time (see below). After removal from the cups, larvae were rinsed in distilled water, blotted dry on tissue paper and immediately frozen in liquid nitrogen and stored at –80°C until analysis.

A preliminary experiment was conducted to measure the time course of incorporation of radiolabel from the bathing solution into the free amino acid pool and the protein pool of the larvae using larvae *ca.* 9 mm TL. These larvae were obtained from a previous spawn by the same red drum broodstock and reared under conditions similar to the experimental fish. For the time-course experiment, groups of larvae were transferred, 24 h after feeding, to incubation cups containing the bathing solution, and left in the dark for 1, 2, 4, 6 and 8 h (three cups sampled at each time point). In addition, a control group was sampled and frozen to provide baseline free amino acid and protein pool data for 9-mm-TL red drum larvae. Based on results of the preliminary time-course experiment (see Results), all subsequent experimental groups were bathed for 4 h. In order to measure post-prandial changes in protein synthesis, groups of larvae were removed from the three stock tanks immediately before feeding (0 h) and 2, 4, 6, 8, 10, 12, 16, 20, 24 and 28 h after feeding and transferred to the incubation cups containing the bathing solution. Three replicate cups per trial were sampled at each time point. These time intervals were selected to capture the expected rapid increase and slow decrease in post-prandial protein synthesis. A failure of air supply to some cups during incubation resulted in the loss of samples for a few time points: 28 h in trial 1 and 16, 20 and 24 h in trial 3.

In order to calculate rates of protein synthesis, the specific radioactivities of the Phe in the free amino acid pool and protein pool were measured as outlined by Houlihan et al. (Houlihan et al., 1995b). Briefly, the frozen samples were homogenised in 0.2 mol l^{-1} perchloric acid and centrifuged (2000 ***g*** at 4°C for 10 min) to separate the precipitated body protein from the free amino acid pools. Larval

protein content (mg larva^{-1}) was measured by solubilising the protein precipitate in 0.3 mol l^{-1} NaOH and determining the protein content using the method of Lowry et al. as modified by Schacter and Pollack using bovine serum albumin as a standard (Lowry et al., 1951; Schacter and Pollack, 1973). The specific radioactivity of Phe in the protein pool (S_b; d.p.m. nmol^{-1} Phe) was calculated from the radioactivity of the solubilised protein (d.p.m. mg^{-1} protein), determined by liquid scintillation counting, divided by the concentration of Phe in the protein (Houlihan et al., 1992; Wilson et al., 1996; Morgan et al., 1998; McCarthy and Fuiman, 2008). We had previously measured the concentration of Phe in red drum larval protein as 348.3±1.9 nmol mg^{-1} (McCarthy and Fuiman, 2008). The specific radioactivity of Phe in the free amino acid pool (S_a; d.p.m. nmol^{-1} Phe) was calculated by converting Phe in the free pool to β-phenylethylamine (PEA), extracting PEA through heptane into 0.01 mol l^{-1} H_2SO_4 and measuring PEA content by fluorescence (Suzuki and Yagi, 1976) and PEA radioactivity using scintillation counting. The enzymic conversion of Phe to PEA is 1:1 (Suzuki and Yagi, 1976). The degree of flooding in the free pool was calculated by comparing the free Phe concentrations in the larvae from the time-course experiment with the average value for the control group.

Daily fractional protein synthesis rates (K_s; % day^{-1}) were calculated as:

$$K_s = [(S_b / S_a) \times (1440 / 240)] \times 100, \quad (1)$$

where 1440 is the number of minutes in a day and 240 is the duration of the bathing period (min) (Garlick et al., 1983). Daily rates of protein synthesis were calculated for each replicate from the mean rate of whole body protein synthesis at each time point measured according to the method of Katersky and Carter (Katersky and Carter, 2010):

$$\text{Daily } K_s = [\Sigma(K_{s,WB,0}\,t + K_{s,WB,2}\,t) + (K_{s,WB,2}\,t + K_{s,WB,4}\,t) + (\ldots) / 24], \quad (2)$$

where $K_{s,WB}$ is the mean whole body fractional rate of protein synthesis at each sampling time and t is the number of hours between the two sampling points multiplied by 0.5.

Absolute rates of protein synthesis (A_s; mg day^{-1}) were calculated from the K_s values as:

$$A_s = (K_s / 100) \times P, \quad (3)$$

where, P is average protein content (mg) of each treatment group. Absolute rates of protein synthesis were calculated for each time point after feeding and hourly absolute rates of protein synthesis (mg h^{-1}) were calculated by dividing these values by 24. The daily A_s value was calculated from the daily K_s value using Eqn 3.

Statistical analyses

Data are presented as means ± 1 s.d. All variables were tested for normality and homogeneity of variances prior to statistical analyses using one-way ANOVA or ordinary least squares linear regression. Statistical analyses were conducted using SPSS v.14 and SYSTAT v.11. Differences present at the 5% level were considered significant.

RESULTS

Method validation

In the method validation experiment, the specific radioactivity of Phe in the intracellular free pool of the larvae was elevated within 1 h after immersion and remained elevated and stable over the 8-h time course (ANOVA, P=0.17; Fig. 1A). The mean specific radioactivity of Phe in the free pool over this time period was 940±168 d.p.m. nmol^{-1} Phe, equivalent to 86.5±17.2% of the specific radioactivity of the bathing solution. The Phe concentration in the free pool of the larvae increased 7.3-fold (±3.9) over the values observed for the initial control larvae at time 0 min. S_b increased linearly over the 8-h time period (Fig. 1B): S_b=1.24×t+3.06 (R^2=0.991, P<0.0001), where t is incubation time (h). These time-course data (Fig. 1) validate the flooding-dose technique for measuring rates of protein synthesis in red drum larvae.

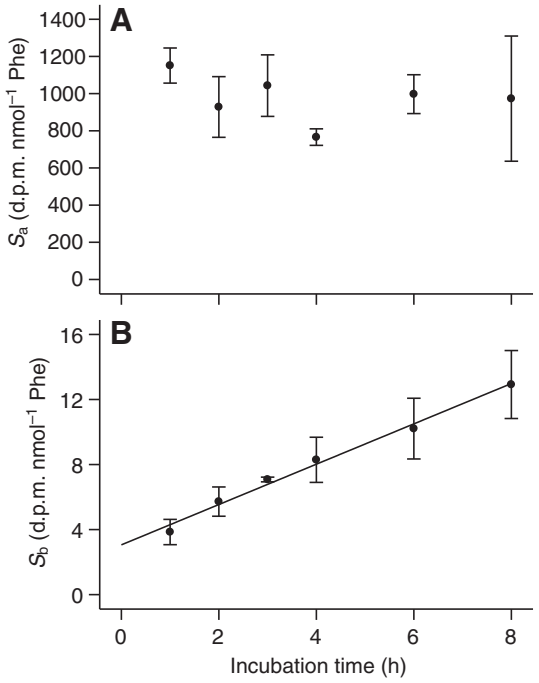

Fig. 1. Validation of the phenylalanine (Phe) flooding-dose technique for measuring rates of protein synthesis in 9 mm TL red drum, *Sciaenops ocellatus*, larvae measured by bathing in a solution containing 135 mmol l^{-1} L-[2,6-^{3}H]Phe (3.7 MBq ml^{-1}; 1087±137 d.p.m. nmol^{-1} Phe). (A) Specific radioactivity of the Phe in the intracellular free amino acid pool (S_a; d.p.m. nmol^{-1} Phe) in relation to time of incubation. (B) Specific radioactivity of the Phe in the body protein pool (S_b; d.p.m. nmol^{-1} Phe) in relation to time of incubation. Values are means ± s.d.

Post-prandial changes in protein synthesis

Larvae from the three trials differed significantly in total length (ANOVA, P<0.0001), body mass (ANOVA, P<0.0001), percentage protein content (ANOVA, P=0.002) and protein mass (ANOVA, P<0.0001; Table 1), so protein synthesis data are presented for each trial separately.

Fractional rates and absolute rates of protein synthesis over the time course varied between trials with values of 10–31% day^{-1} and 25–81 μg protein day^{-1} in trial 1, 8–56% day^{-1} and 39–270 μg protein day^{-1} in trial 2, and 19–56% day^{-1} and 61–182 μg protein day^{-1} in trial 3. Although there were differences between trials in the pre-feeding and maximum post-feeding rates of protein synthesis and in the timing of the maximum rate, the same post-prandial pattern was present in each trial. To compare results between the three separate trials, fractional protein synthesis rates for each trial were standardised to a common baseline by dividing each K_s value in a trial by the K_s value for the sample taken just before feeding (i.e. 0 h). This expresses each K_s value in terms of the change relative to the pre-feeding baseline (K_s=1). The combined time-course data are presented in Fig. 2. This analysis

Table 1. Characteristics of fish used for the protein synthesis measurements

Trial	Number of fish per sample	Total length (mm)	Body mass (mg)	Protein mass (mg larva^{-1})	% Protein
1	27.7±2.4 (25–30)	7.4±0.5^{a} (6.6–8.2)	3.4±0.6^{a} (2.4–4.4)	0.259±0.052 (0.176–0.355)	7.8±0.6^{a,b} (6.8–8.8)
2	18.2±1.8 (17–21)	8.9±0.4 (8.3–9.5)	5.6±0.6 (4.6–6.7)	0.481±0.057 (0.413–0.577)	8.5±0.4^{a} (7.7–9.1)
3	26.6±3.1 (22–30)	7.8±0.3^{a} (7.3–8.3)	3.9±0.4^{a} (3.2–4.5)	0.327±0.043 (0.258–0.382)	8.4±0.4^{b} (8.0–9.0)

Values are means ± s.d. for each trial with the range given in parentheses. Mean values with the same superscript letter (within columns) are similar (P>0.05).

indicated that the highest fractional rates of protein synthesis occurred 4–12 h after feeding, with a post-prandial peak in protein synthesis *ca.* 8 h after feeding (Fig. 2). During this peak period of protein synthesis, fractional rates averaged 2- to 2.5-fold higher than pre-feeding rates. Fractional rates of protein synthesis returned to pre-feeding levels at approximately 16 h and possibly decreased further, reaching a minimum value 24 h after feeding. It is interesting to note a slight increase in protein synthesis from this minimum value 28 h after feeding. The peak period of protein synthesis (4–12 h after feeding) accounted for 56–63% of the total protein synthesised in the 24 h following the meal with 7–8% of this daily total synthesised per hour at the post-prandial peak (Fig. 3).

DISCUSSION

Validation of flooding-dose method

This study represents a successful modification of the flooding-dose technique to assess post-prandial changes in protein synthesis in regularly fed larval red drum. Requirements of the flooding-dose technique are: (1) rapid elevation of specific radioactivity of Phe in the body free amino acid pools following administration of the radiolabel; (2) the specific radioactivity of Phe in the body free amino acid pools should remain elevated and stable or show a slow linear decline over time; (3) linear incorporation of radiolabel into body protein over time; and (4) no effect on the rate of protein synthesis by the presence of the high intracellular free Phe concentration (Houlihan et al., 1995a; Houlihan et al., 1995b). The preliminary time-course experiment showed that the first three criteria were met for red drum larvae of *ca.* 9 mm TL. Previously, we validated this method for red drum larvae of 11 mm TL (McCarthy and Fuiman, 2008) and 5 and 20 mm TL (I.D.M. and L.A.F., unpublished results). Furthermore, flooding doses of Phe are not thought to affect rates of protein synthesis (criterion 4) (Garlick et al., 1980; Loughna and Goldspink, 1985). In the present study, the specific radioactivity of Phe in the body free amino acid pool was elevated within 1 h of bathing and remained elevated and stable during the 8-h time course (Fig. 1A). Rapid elevation and stability of the specific radioactivity of Phe in the free amino acid pool within 1 to 2 h of bathing has been observed in most studies of protein synthesis of larval fishes (Houlihan et al., 1992; Houlihan et al., 1995d; Smith and Ottema, 2006; McCarthy and Fuiman, 2008) (this study) although in some rapidly growing fish larvae this has not been achieved (Conceição et al., 1997a; Conceição et al., 1997b). In the present study, the free Phe concentration in the red drum larvae was elevated sevenfold over the baseline level recorded in control larvae. This compares favourably with previous levels of intracellular free Phe elevation reported for red drum (10-fold) (McCarthy and Fuiman, 2008) and lies within the range of values typically recorded in studies using the flooding-dose technique (4- to 10-fold) (Fraser and Rogers, 2007).

Post-prandial stimulation

Our results traced the post-prandial increase in whole-body rates of protein synthesis in regularly fed red drum larvae. Compared with baseline (pre-feeding) levels, rates of protein synthesis increased to a peak *ca.* 8 h after feeding then declined to baseline or lower values *ca.* 24 h after feeding (Fig. 2). It is interesting that there was a slight increase in rates of protein synthesis after 24 h, which coincided with the scheduled daily meal. This rise in protein synthesis may be due to the synthesis of digestive enzymes or ribosomes in anticipation of the next meal. In fishes reared under feeding

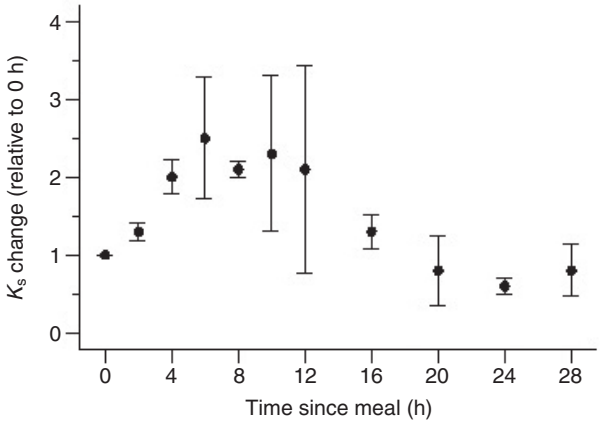

Fig. 2. Time course of fractional rates of protein synthesis (K_s; % day^{-1}) in red drum larvae over a 28 h time period in three trials where food (*Artemia*, 5 nauplii ml^{-1}) was provided as a single meal at time 0 h. Data are expressed as means ± s.d. K_s values for each trial were baseline-adjusted.

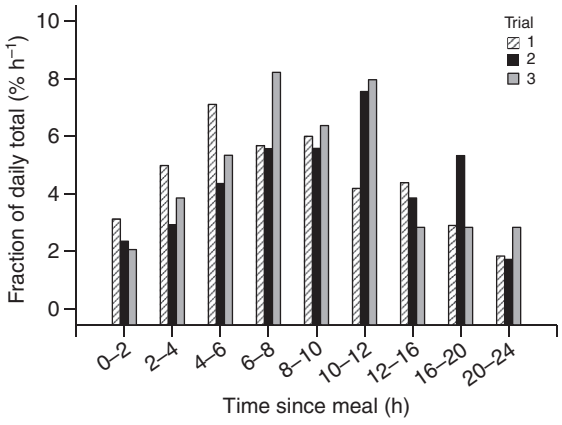

Fig. 3. The percentage contribution per hour towards the total amount of protein synthesised over a 24 h time period in the three trials where food (*Artemia*, 5 nauplii ml^{-1}) was provided as a single meal at time 0 h. The 24 h time period is divided into a series of 2 h (0–12 h after feeding) and 4 h (12–24 h after feeding) intervals. Hatched bars, trial 1; black bars, trial 2; grey bars, trial 3.

schedules where the daily ration is always offered at the same time of day, feeding entrainment results in diurnal variation in the production of digestive enzymes with a pre-feeding anticipatory rise in digestive enzyme activities 2–6 h before the regular feeding (Vera et al., 2007; Montoya et al., 2010). Diurnal (Mackenzie et al., 1999; Fujii et al., 2007) and post-prandial (Gárcia-Ortega et al., 2000) variation in digestive enzyme activities have also been reported for fish larvae. In addition, Rooker and Holt have shown that there is diel variation in RNA:DNA ratios in larval red drum, with high ratios during the daytime and low ratios at night (Rooker and Holt, 1996). Although diel periodicities in digestive enzyme activity and RNA:DNA ratios have been linked to photoperiod because most fish larvae are visual feeders (Rooker and Holt, 1996; Fujii et al., 2007), it has also been suggested that digestive enzyme activities may also show an endogenous circadian rhythm, where peaks in activity anticipate feeding activity, ingestion and digestion of food (Fujii et al., 2007).

The timing and magnitude of the post-prandial peak in protein synthesis varied between trials in the present study. This variability is not unexpected, as previous studies with red drum have shown variation in physiological and behavioural performance between trials and batches of fish (Fuiman et al., 2005; McCarthy and Fuiman, 2008). Such differences have been attributed to variation in the quality of eggs produced at different times within the spawning cycle of individual females within the broodstock (Fuiman et al., 2005). In addition, it is likely that differences in meal size between trials may have influenced the timing and magnitude of the post-prandial peak in protein synthesis (McCarthy et al., 1994; Carter et al., 2001).

Post-prandial protein metabolism has been studied in a number of fish species encompassing a range of body sizes, temperatures, tissue/organ types and feeding regimens. All of these studies show the same pattern of response: a post-prandial increase in protein synthesis to a peak followed by a subsequent decline (Fig. 4). The timing and magnitude of the post-prandial peak, however, can vary between tissues and feeding regimens. Post-prandial peaks in protein synthesis occur earlier and are of greater magnitude in the liver than in other tissues. In regularly fed animals, peak tissue-level and whole-body rates of protein synthesis occur 4–10 h after feeding and involve a 2- to 2.5-fold increase in synthesis rates over pre-feeding levels (Fig. 4A,C). In addition, the results of Lyndon et al. suggest that the magnitude of the post-prandial response may be larger in animals fed a single meal after extended starvation (Lyndon et al., 1992) (Fig. 4D). The rapid rise in rates of protein synthesis in the liver is to be expected given the primary role of the liver in assimilating nutrients derived from feeding. Indeed, post-prandial rates of protein synthesis are thought to be the driving force behind the SDA response (reviewed by Katersky and Carter, 2010). In barramundi (*Lates calcarifer*), it was shown (Katersky and Carter, 2010) that the post-prandial profile of whole-body fractional rates of protein synthesis very closely matches the response of the liver, and at the peak post-prandial response 4 h after feeding, the liver appears to be synthesising 20 times more protein (on an absolute basis) than the entire white muscle. Thus, it is likely that the timing and magnitude of the post-prandial response of whole-body rates of protein synthesis observed in larval fishes (Houlihan et al., 1993) (this study) are driven by the post-prandial response in the liver.

Based on work on larger fishes (>10 g), it is thought that the post-prandial increase in protein synthesis is driven by increases in the rate of ribosomal translation, as indicated by calculation of K_{RNA} (also known as RNA activity), rather than by increases in ribosomal number, as indicated by the RNA:protein ratio (also known as C_s, capacity for protein synthesis) (Houlihan et al., 1988; Katersky and Carter, 2010). Changes in ribosomal number are thought to be a long-term acclimatory response to changing conditions (e.g. temperature, food supply) in fishes, whereas short-term responses are managed by changes in the rate at which existing ribosomes produce proteins (Fraser and Rogers, 2007; Katersky and Carter, 2010). However, Rooker and Holt have shown that there is diel variation in RNA:DNA ratios in larval red drum (6–20 mm; age 16–40 days; 26°C), with high ratios during the daytime and low ratios at night (Rooker and Holt, 1996). Thus, post-prandial changes in protein synthesis in larval fishes may be a result of both changes in ribosomal number and activity, possibly driven by circadian periodicities in endocrine substances, such as growth-regulating hormones (Rooker and Holt, 1996). In addition, insulin also plays a role in the timing and magnitude of the post-prandial increase in

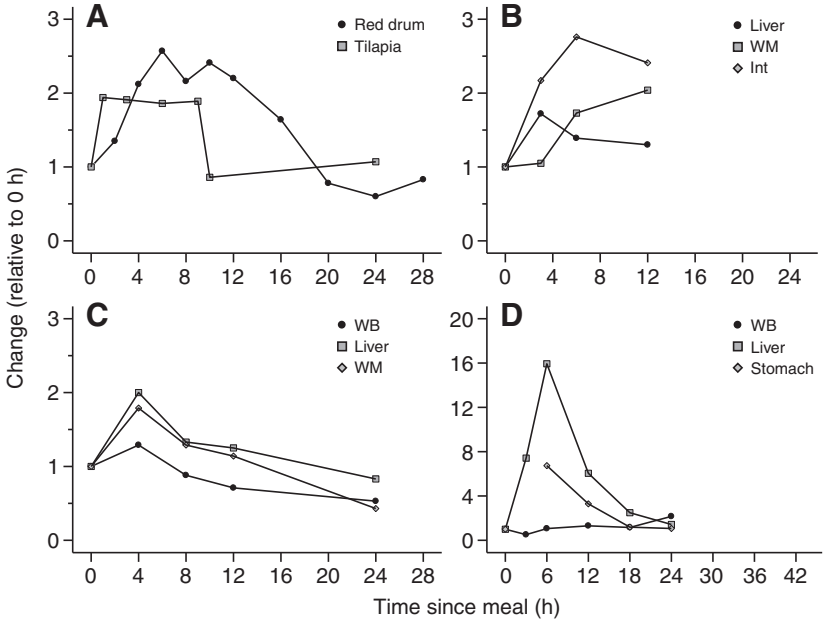

Fig. 4. Post-prandial changes in fractional rates of protein synthesis in various fish species, expressed as the change in rate relative to the rate recorded immediately before feeding. (A) Regularly fed fish larvae [tilapia, *Oreochromis mossambicus*, 18 mg, 27°C (Houlihan et al., 1993); red drum, *Sciaenops ocellatus*, 4 mg, 28°C (present study)]. (B) Rainbow trout, *Oncorhynchus mykiss*, fed a single meal after a 6 day starvation period [50 g, 28°C (Houlihan et al., 1988)]. (C) Regularly fed barramundi, *Lates calcarifer* [11 g, 27°C, (Katersky and Carter, 2010)]. (D) Atlantic cod, *Gadus morhua*, fed a single meal after a 6 day starvation period [180 g, 10°C (Lyndon et al., 1992)]. Int, intestine; WB, whole body; WM, white muscle.

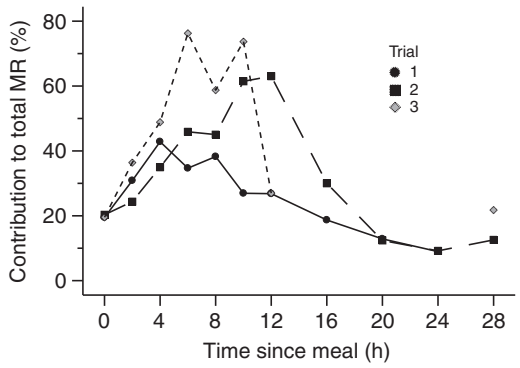

Fig. 5. The percentage contribution of protein synthesis to total oxygen consumption (metabolic rate; MR) in red drum larvae over a 28 h time period in three trials where food (*Artemia*, 5 nauplii ml^{-1}) was provided as a single meal at time 0 h. A protein synthesis cost-factor of 12.3 mmol O_2 g^{-1} protein synthesised was used as this value is based on experimental data rather than a minimum cost based on theoretical assumptions [see reviews by Houlihan et al. (Houlihan et al., 1995a; Houlihan et al., 1995b) or Fraser and Rodgers (Fraser and Rodgers, 2007) for assumptions].

protein synthesis in mammals (Waterlow, 2006) and although not yet investigated in fishes, is likely to play a similar role in the regulation of protein turnover in fishes.

Although several studies have reported the post-prandial response of protein synthesis in fishes, our understanding of the timing and the magnitude of the peak response and the overall profile of the response over time after a meal has been limited by experimental design. Firstly, many studies have adopted the starvation followed by re-feeding approach (Houlihan et al., 1988; McMillan and Houlihan, 1988; Fauconneau et al., 1989; Lyndon et al., 1992) rather than using regularly fed animals (Houlihan et al., 1993; Katersky and Carter, 2010). Although starvation–re-feeding studies can provide valuable information on the maximum response of tissue and/or whole-body synthesis rates following food deprivation, responses observed in regularly fed animals are more valuable for understanding protein metabolism and energetics in fishes. Secondly, previous studies have made a limited number of measurements of protein synthesis in order to describe the post-prandial response in the following 24 h (e.g. Houlihan et al., 1988; Fauconneau et al., 1989; McMillan and Houlihan, 1989; McMillan and Houlihan, 1992; Lyndon et al., 1992; Katersky and Carter, 2010). These studies have described the general post-prandial response (Fig. 4); however, the precise timing and magnitude of the post-prandial peak in protein synthesis may have been missed. The larval fish studies (Houlihan et al., 1993) (this study) that included more frequent sampling during the first 12 h after feeding, provide more precise estimates of the timing and magnitude of the post-prandial peak in protein synthesis. In addition, our study is the first to suggest that rates of protein synthesis fall below baseline levels and then rise at the end of the 24 h period, possibly in anticipation of the next meal.

Protein synthesis and energetics

The mean daily rate of protein synthesis in the present study was 26.9% day^{-1} for the 4.3 mg (range 2.4–6.7 mg) red drum larvae (Table 2). These daily fractional rates of protein synthesis are similar to values previously reported for red drum larvae (3–22 mg; 27°C, 10–24% day^{-1}) (McCarthy and Fuiman, 2008) and for other small exogenously feeding larvae of warm-water fish species: nase, *Chondrostoma nasus* (30–40 mg; 20°C, 30% day^{-1}) (Houlihan et al., 1992) and tilapia, *Oreochromis mossambicus* (16 mg; 27°C, 29.5% day^{-1}) (Houlihan et al., 1993). Although some studies have reported larval protein synthesis rates of 100–600% day^{-1}, these extreme values have been questioned (reviewed in Smith and Ottema, 2006). Available flooding-dose data would suggest that whole-body rates of protein synthesis in larval fishes (10^{-2}–10^{-3} g) are an order of magnitude higher than in larger juvenile and adult fishes (10^{1}–10^{3} g): *ca.* 30% day^{-1} compared with 1–5% day^{-1} depending upon temperature and feeding regimen (Houlihan et al., 1995a; Houlihan et al., 1995b; Carter and Houlihan, 2001) [although see Katersky and Carter (Katersky and Carter, 2010)]. It has been suggested that protein synthesis rates in excess of *ca.* 30% day^{-1} are energetically impossible in larval poikilotherms (Weiser, 1991). However, our results show that rates in excess of 30% day^{-1} can be attained by larval fishes for periods of 4–8 h but perhaps not sustained for longer periods of time. During the 24 h after feeding, fractional rates of protein synthesis in red drum larvae varied from baseline rates equivalent to an average of 15.7% day^{-1} (range 14–18% day^{-1}) immediately before feeding, rising to an average post-prandial peak of 47.7% day^{-1} (range 32–56% day^{-1}) and declining to an average daily rate of 12.3% day^{-1} (range 9–20% day^{-1}) 24–28 h after feeding. Expressed as absolute rates of protein synthesis (g protein h^{-1}) these values indicate that 56–63% of the total protein synthesised in the 24-h period occurred during the peak period of protein synthesis 4–12 h after feeding.

Protein synthesis is known to account for a significant proportion of an animal's energy budget (Fraser and Rodgers, 2007) and in fishes accounts for 11–49% of total energy expenditure (Houlihan et al., 1995a; Carter and Houlihan, 2001). Three approaches have been used to determine the energetic cost of protein synthesis: (1) multiplying the measured rate of protein synthesis by an energetic cost factor; (2) deriving the regression relationship between measured rates of oxygen consumption and protein synthesis; or

Table 2. A summary of the daily rates of protein synthesis for juvenile red drum (reared at 27.4°C, 27.7 PSU) expressed on a fractional (K_s) and an absolute (A_s) basis, and the percentage contribution made by protein synthesis towards daily energy expenditure

					% Total daily O_2 consumption‡	
Trial	Wet body mass (mg)	Dry body mass (mg)	K_s*	A_s†	At 8.3 mmol g^{-1}	At 12.3 mmol g^{-1}
1	3.4	0.52	19.7	0.052	17.3	25.7
2	5.6	0.98	28.4	0.144	24.8	36.8
3	3.9	0.63	32.6	0.094	25.8	38.3

*Percentage protein mass synthesised day^{-1}.

†mg protein synthesised day^{-1}.

‡As converted to μg O_2 larva^{-1} day^{-1} assuming theoretical minimum costs of 8.3 or 12.3 mmol O_2 g^{-1} protein synthesised (see text for explanation). Daily total metabolic rate (μg O_2 larva^{-1} day^{-1}) was calculated using the equation provided by Torres et al. (Torres et al., 1996): $Y=4.58X^{1.04}$ where X is dry body mass (mg) and Y is total metabolic rate (μl O_2 larva^{-1} h^{-1}).

(3) measuring the rates of protein synthesis and oxygen consumption in the presence and absence of a protein synthesis inhibitor (Houlihan et al., 1995a; Houlihan et al., 1995b; Fraser and Rodgers, 2007). The theoretical minimum cost of protein synthesis, derived from stoichiometry is 8.3 mmol O_2 g^{-1} protein synthesised [see reviews by Houlihan et al. (Houlihan et al., 1995a; Houlihan et al.,1995b) or Fraser and Rodgers (Fraser and Rodgers, 2007) for assumptions]. Several studies have used protein synthesis inhibitors to estimate the energetic cost of protein synthesis in fish cells and fast-growing fish larvae. The results of these studies [summarised by Smith and Ottema (Smith and Ottema, 2006)] suggest that the relationship between the absolute rate of protein synthesis and the cost of protein synthesis is a declining exponential curve [fig. 7 in Smith and Ottema (Smith and Ottema, 2006)]. The energetic cost of protein synthesis appears to reach a minimal value of 12.3±2.2 mmol O_2 g^{-1} protein synthesised at high absolute rates of protein synthesis (>3 mg protein synthesised g^{-1} protein h^{-1}) [derived from data provided by Smith and Houlihan (Smith and Houlihan, 1995), Smith et al. (Smith et al., 2001a; Smith et al., 2001b) and Smith and Ottema (Smith and Ottema, 2006)]. We estimated the cost of protein synthesis for larval red drum from the absolute rates of protein synthesis using protein synthesis cost-factors of 8.3 and 12.3 mmol O_2 g^{-1} protein synthesised (Table 2). The result was an average daily energetic cost of protein synthesis in regularly fed red drum larvae of between 23 and 34% of the estimated daily total oxygen consumption [calculated from the dry mass using the equation of Torres et al.; $Y=4.58X^{1.04}$, where Y is total metabolic rate (in µl O_2 $larva^{-1}$ h^{-1}) and X is the dry body mass (in mg) to estimate rates of oxygen consumption (Torres et al., 1996)] depending on the cost-factor used. This analysis can be extended to determine the contribution of protein synthesis to hourly rates of oxygen consumption (µl O_2 $larva^{-1}$ h^{-1}; Fig. 5). Using a cost-factor of 12.3 mmol O_2 g^{-1} protein synthesised, the results indicated that protein synthesis used 19.1±0.2% of the total oxygen consumption immediately before feeding. After feeding, the contribution of protein synthesis increased to a post-prandial peak of 60.7±15.9% (range 43.4–75.4%) then declined to a minimum of 13.5±4.8% (range 9.4–22.5%) 24–28 h after feeding (Fig. 5).

It is important to stress the speculative nature of our conclusions since our analysis uses literature-derived average values to estimate metabolic rate [based on Torres et al. (Torres et al., 1996)] and cost of protein synthesis (based on a summary of published data). Inter-spawn differences in physiological performance are known to exist (McCarthy and Fuiman, 2008), so that both daily metabolic rate and the cost of protein synthesis are likely to have varied between our replicates. However, these preliminary results do suggest that during the post-prandial peak, protein synthesis will require a large proportion of the hourly energy production, and this is an area of research that warrants further investigation. It is probable that, given the limited metabolic scope in fish larvae (Lucas and Priede, 1992), any short-term energy requirement for protein synthesis will limit the energy that can be allocated to other functions, such as foraging or predator evasion. Reduced activity after feeding has been observed in a number of taxa, e.g. fishes (Owen, 2001; Vera et al., 2007; Sánchez et al., 2009; Montoya et al., 2010) and reptiles (Secor and Diamond, 1997) and has been suggested for larval fishes based on oxygen consumption data (Wieser et al., 1988; Cunha et al., 2007). However, changes in activity or performance levels after feeding have not been directly related to post-prandial changes in metabolic rate and any effects of reduced activity levels or reduced energy availability for locomotor activities such as escape responses (McCarthy et al., 2003; Fuiman et al., 2005) on predation susceptibility in larval fishes have yet to be determined.

ACKNOWLEDGEMENTS

We thank Hunter Samberson for conducting the experiments and performing the biochemical analyses. Dr Maria Alvarez and Brie Sarkisian helped with the fish husbandry. We are grateful to the referees and editor for their comments on the draft manuscript. This research was funded by the Texas Sea Grant program (grant number NA16RG1078) and the University of Texas at Austin. Contribution number 1580 of the University of Texas Marine Science Institute.

REFERENCES

Arnold, C. R. (1988). Controlled year-round spawning of red drum (*Sciaenops ocellatus*) in captivity. *Contrib. Mar. Sci.* **30 Suppl**, 65-70.

Carter, C. G. and Houlihan, D. F. (2001). Protein synthesis. In *Fish Physiology Vol. 20 Nitrogen Excretion* (ed. P. A. Wright and P. M. Anderson), pp. 31-75. London: Academic Press.

Carter, C. G., Houlihan, D. F., Kiessling, A., Medale, F. and Jobling, M., (2001). Physiological effects of feeding. In *Food Intake in Fishes* (ed. D. F. Houlihan, T. Boujard and M. Jobling), pp. 297-331. Oxford: Blackwell Scientific.

Conceição, L. E. C., Houlihan, D. F. and Verreth, J. A. J. (1997a). Fast growth, protein turnover and costs of protein metabolism in yolk-sac larvae of the African catfish (*Clarias gariepinus*). *Fish Physiol. Biochem.* **16**, 291-302.

Conceição, L. E. C., Van der Meeren, T., Verreth, J. A. J., Evjen, M. S., Houlihan, D. F. and Fyhn, H. J. (1997b). Amino acid metabolism and protein turnover in larval turbot (*Scopthalmus maximus*) fed natural zooplankton or *Artemia*. *Mar. Biol.* **129**, 255-265.

Cunha, I., Conceição, L. E. C. and Planas, M. (2007). Energy allocation and metabolic scope in early turbot, *Scophthalmus maximus*, larvae. *Mar. Biol.* **151**, 1397-1405.

Danicke, S., Nieto, R., Lobley, G. E., Fuller, M. F., Brown, D. S., Milne, E., Calder, A. G., Chen, S., Grant, I. and Bottcher, W. (1999). Responses in the absorptive phase in muscle and liver protein synthesis rates of growing rats. *Arch. Anim. Nutr.* **52**, 41-52.

Fauconneau, B., Breque, J. and Bielle, C. (1989). Influence of feeding on protein metabolism in Atlantic salmon (*Salmo salar*). *Aquaculture* **79**, 29-36.

Fraser, K. P. P. and Rogers, A. D. (2007). Protein metabolism in marine animals: the underlying mechanism of growth. *Adv. Mar. Biol.* **52**, 267-362.

Fuiman, L. A. (2002). Special considerations of fish eggs and larvae. In *Fishery Science: The Unique Contribution of Early Life Stages* (ed. L. A. Fuiman and R. G. Werner), pp. 1-32. Oxford: Blackwell Science.

Fuiman, L. A., Poling, K. R. and Higgs, D. M. (1998). Quantifying developmental progress for comparative studies of larval fishes. *Copeia* **1998**, 602-611.

Fuiman, L. A., Cowan, J. H., Jr, Smith, M. E. and O'Neal, J. P. (2005). Behavior and recruitment success in fish larvae: variation with growth rate and the batch effect. *Can. J. Fish. Aquat. Sci.* **62**, 1337-1349.

Fujii, A., Kurokawa, Y., Kawai, S., Yoseda, K., Dan, S., Kai, A. and Tanaka, M. (2007). Diurnal variation of tryptic activity in larval stage and development of proteolytic enzyme activities of Malabar grouper (*Epinephelus malabaricus*) after hatching. *Aquaculture* **270**, 68-76.

Gárcia-Ortega, A., Verreth, J. and Segner, H. (2000). Post-prandial protease activity in the digestive tract of African catfish *Clarias gariepinus* larvae fed decapsulated cysts of *Artemia*. *Fish Physiol. Biochem.* **22**, 237-244.

Garlick, P. J., McNurlan, M. A. and Preedy, V. A. (1980). A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of ^{3}H phenylalanine. *Biochem. J.* **192**, 719-723.

Garlick, P. J., Fern, M. and Preedy, V. A. (1983). The effect of insulin infusion and food intake on muscle protein synthesis in postabsorptive rats. *Biochem. J.* **210**, 669-676.

Herzka, S. Z. and Holt, G. J. (2000). Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. *Can. J. Fish. Aquat. Sci.* **57**, 137-147.

Houlihan, D. F. (1991). Protein turnover in ectotherms and its relationships to energetics. *Adv. Comp. Environ. Physiol.* **7**, 1-43.

Houlihan, D. F., Hall, S. J., Gray, C. and Noble, B. S. (1988). Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. *Can. J. Fish. Aquat. Sci.* **45**, 951-964.

Houlihan, D. F., Waring, C. P., Mathers, E. and Gray, C. (1990). Protein synthesis and oxygen consumption of the shore crab *Carcinus maenas* after a meal. *Physiol. Zool.* **63**, 735-756.

Houlihan, D. F., Wieser, W., Foster, A. and Brechin, J. (1992). In vivo protein synthesis rates in larval nase (*Chondrostoma nasus* L.). *Can. J. Zool.* **70**, 2436-2440.

Houlihan, D. F., Pannevis, M. C. and Heba, H. (1993). Protein synthesis in juvenile tilapia *Oreochromis mossambicus*. *J. World Aquac. Soc.* **24**, 145-151.

Houlihan, D. F., Carter, C. G. and McCarthy, I. D. (1995a). Protein turnover in animals. In *Nitrogen Metabolism and Excretion* (ed. P. J. Walsh and P. Wright), pp. 1-32. Boca Raton, FL: CRC Press.

Houlihan, D. F., Carter, C. G. and McCarthy, I. D. (1995b). Protein synthesis in fishes. In *Biochemistry and Molecular Biology of Fishes, Vol. 4, Metabolic Biochemistry* (ed. P. Hochachka and T. P. Mommsen), pp. 191-220. Amsterdam: Elsevier Science.

Houlihan, D. F., McCarthy, I. D., Carter, C. G. and Marttin, F. (1995c). Protein turnover and amino acid flux in fish larvae. *ICES. Mar. Sci. Symp.* **201**, 87-99.

Houlihan, D. F., Pedersen, B. H., Steffensen, J. F. and Brechin, J. (1995d). Protein synthesis, growth and energetics in larval herring (*Clupea harengus*) at different feeding regimes. *Fish Physiol. Biochem.* **14**, 195-208.

Katersky, R. S. and Carter, C. G. (2010). The effect of temperature on post-prandial protein synthesis in juvenile barramundi *Lates calcarifer*. *Comp. Biochem. Physiol.* **156A**, 529-536.
Loughna, P. T. and Goldspink, G. (1985). Muscle protein synthesis rates during temperature acclimation in a eurythermal (*Cyprinus carpio*) and a stenothermal (*Salmo gairdneri*) species of teleost. *J. Exp. Biol.* **118**, 267-276.
Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
Lucas, M. C. and Priede, I. C. (1992). Utilization of metabolic scope in relation to feeding and activity by individual and grouped zebrafish, *Brachydanio reria* (Hamilton-Buchanan). *J. Fish Biol.* **41**, 175-190.
Lyndon, A. R., Houlihan, D. F. and Hall, S. J. (1992). The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. *J. Comp. Physiol. B* **162**, 209-215.
MacKenzie, B. R., Ueberschär, B., Basford, D., Heath, M. and Gallego, A. (1999). Diel variability of feeding activity in haddock (*Melanogrammus aeglifinus*) larvae in the East Shetland area, North Sea. *Mar. Biol.* **135**, 361-368.
Mathers, E., Houihan, D. F., McCarthy, I. D. and Burren, L. J. (1993). Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*: effects of age and temperature. *J. Fish Biol.* **43**, 245-263.
McCarthy, I. D. and Fuiman, L. A. (2008). Growth and protein metabolism in red drum (*Sciaenops ocellatus*) larvae exposed to environmental levels of Atrazine and Malathion. *Aquat. Toxicol.* **88**, 220-229.
McCarthy, I. D., Houlihan, D. F. and Carter, C. G. (1994). Individual variation in protein turnover and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Proc. R. Soc. Lond. B* **257**, 141-147.
McCarthy, I. D., Fuiman, L. A. and Alvarez, M. C. (2003). Aroclor 1254 affects growth and survival skills of Atlantic croaker (*Micropogonias undulatus*) larvae. *Mar. Ecol. Progr. Ser.* **252**, 295-301.
McCue, M. D. (2006). Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol.* **144A**, 381-394.
McMillan, D. N. and Houlihan, D. F. (1988). The effect of refeeding on tissue protein synthesis in rainbow trout. *Physiol. Zool.* **61**, 429-441.
McMillan, D. N. and Houlihan, D. F. (1989). Short-term responses of protein synthesis to re-feeding in rainbow trout. *Aquaculture* **79**, 37-46.
McMillan, D. N. and Houlihan, D. F. (1992). Protein synthesis in trout liver is stimulated by both feeding and fasting. *Fish Physiol. Biochem.* **10**, 23-34.
McNurlan, M. A., Essen, P., Milne, E., Vinnars, E. and Garlick, P. J. (1993). Temporal responses of protein synthesis in human skeletal muscle to feeding. *Br. J. Nutr.* **69**, 117-126.
Montoya, A., López-Olmeda, J. F., Yúfera, M., Sánchez-Muros, M. J. and Sánchez-Vázquez, F. J. (2010). Feeding time synchronises daily rhythms of behavior and digestive physiology in gilthead seabream (*Sparus aurata*). *Aquaculture* **306**, 315-321.
Morgan, I. J., D'Cruz, L. M., Dockray, J. J., Linton, T. K., McDonald, D. G. and Wood, C. M. (1998). The effects of elevated winter temperature and sub-lethal pollutants (low pH, elevated ammonia) on protein turnover in the gill and liver of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **19**, 377-389.
Negatu, Z. and Meier, A. H. (1993). Daily variation of protein synthesis in several tissues of the gulf killifish, *Fundulus grandis* Baird and Girard. *Comp. Biochem. Physiol.* **106A**, 251-255.
Owen, S. F. (2001). Meeting energy budgets by modulation of behaviour and physiology in the eel (*Anguilla anguilla* L.). *Comp. Biochem. Physiol.* **128A**, 631-644.
Pedersen, B. H. (1997). The cost of growth in young fish larvae: a review of new hypotheses. *Aquaculture* **155**, 259-269.
Reeds, P. J., Fuller, M. F. and Nicholson, B. A. (1985). Metabolic basis of energy expenditure with particular reference to protein. In *Substrate and Energy Metabolism in Man* (ed. J. S. Garrow and D. Halliday), pp. 46-57. London: John Libbey.
Rooker, J. R. and Holt, G. J. (1996). Application of RNA:DNA ratios to evaluate the condition and growth of larval and juvenile red drum (*Sciaenops ocellatus*). *Mar. Freshw. Res.* **47**, 283-290.
Sánchez, J. A., López-Olmeda, J. F., Blanco-Vives, B. and Sánchez-Vázquez, F. J. (2009). Effects of feeding schedule on locomotor activity rhythms and stress response in sea bream. *Physiol. Behav.* **98**, 125-129.
Schacter, G. R. and Pollack, R. L. (1973). Simplified method for quantitative assay of small amounts of protein in biologic material. *Anal. Biochem.* **51**, 654-655.
Secor, S. M. (2009). Specific dynamic action: a review of the post-prandial metabolic response. *J. Comp. Physiol. B* **179**, 1-56.
Secor, S. M. and Diamond, J. (1997). Effects of meal size on postprandial responses in juvenile Burmese pythons (*Python molurus*). *Am. J. Physiol.* **272**, R902-R912.
Simon. O. and Bergner, H. (1983). Postprandial changes of the rate of protein synthesis in liver and small intestine of rats. *Biomed. Biochim. Acta* **42**, 1299-1308.
Smith, R. W. and Houlihan, D. F. (1995). Protein synthesis and oxygen consumption in fish cells. *J. Comp. Physiol.* **165**, 93-101.
Smith, R. W. and Ottema, C. (2006). Growth, oxygen consumption, and protein and RNA synthesis rates in the yolk-sac larvae of the African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* **143A**, 315-325.
Smith, R. W., Blaney, S. C., Dowling, K., Sturm, A., Jönsson, M. and Houlihan, D. F. (2001a). Protein synthesis costs could account for the tissue-specific effects of sub-lethal copper on protein synthesis in rainbow trout. *Aquat. Toxicol.* **53**, 265-277.
Smith, R. W., Jönsson, M., Houlihan, D. F. and Pärt, P. (2001b). Minimising aerobic respiratory demands could form the basis of sub-lethal copper tolerance by rainbow trout gill epithelial cells in vitro. *Fish Physiol. Biochem.* **24**, 157-169.
Suzuki, O. and Yagi, K. (1976). A fluorometric assay for phenylethylamine in rat brain. *Anal. Biochem.* **75**, 192-200.
Torres, J. J., Brightman, R. I., Donnelly, J. and Harvey, J. (1996). Energetics of larval red drum *Sciaenops ocellatus*. Part I: oxygen consumption, specific dynamic action and nitrogen excretion. *Fish. Bull.* **94**, 756-765.
Vera, L. M., De Pedro, N., Gómez-Milán, E., Delgado, M. J., Sánchez-Muros, M. J., Madrid, J. A. and Sánchez-Vázquez, F. J. (2007). Feeding entrainment of locomotor activity rhythms, digestive enzymes and neuroendocrine factors in goldfish. *Physiol. Behav.* **90**, 518-524.
Waterlow, J. C. (2006). *Protein Turnover*, 320 pp. Oxford: CAB International.
Welle, S., Thornton, C., Statt, M. and McHenry, B. (1994). Postprandial myofibrillar and whole-body protein synthesis in young and old human-subjects. *Am. J. Physiol.* **267**, E599-E604.
Whiteley, N. M., Robertson, R. F., Meagor, J., El Haj, A. J. and Taylor, E. W. (2001). Protein synthesis and specific dynamic action in crustaceans: effects of temperature. *Comp. Biochem. Physiol.* **128A**, 595-606.
Wieser, W. (1991). Essay review: limitations of energy acquisition and energy use in small poikilotherms: evolutionary implications. *Funct. Ecol.* **5**, 234-240.
Wieser, W. (1995). Energetics of fish larvae, the smallest vertebrates. *Acta Physiol. Scand.* **154**, 279-290.
Wieser, W., Forstner, H., Medgyesy, N. and Hinterleitner, S. (1988). To switch or not to switch: partitioning of energy between growth and activity in larval cyprinids (Cyprinidae: teleostei). *Funct. Ecol.* **2**, 499-507.
Wilson, R. W., Wood, C. M. and Houlihan, D. F. (1996). Growth and protein turnover during acclimation to acid and aluminium in juvenile rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **53**, 802-811.
Yaman, M. A., Kita, K. and Okumura, J. (2000). Different responses of protein synthesis to refeeding in various muscles of fasted chicks. *Br. Poult. Sci.* **41**, 224-228.
Yoshizawa, F., Endo, M., Ide, H., Yagasaki, K. and Funabiki, R. (1995). Translational regulation of protein synthesis in the liver and skeletal muscle of mice in response to refeeding. *J. Nutr. Biochem.* **6**, 130-136.