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RESEARCH ARTICLE

A comparison of absorption and assimilation efficiencies between four species of shallow- and deep-living fishes

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Abstract We captured two species of deep-sea zoarcids, *Melanostigma pammelas* and *Lycodapus mandibularis*, from Monterey Bay California and maintained them in the laboratory. One shallow-water zoarcid, *Eucryphycus californicus*, and an ecologically and morphologically similar stichaeid fish *Xiphister atropurpureus* were collected from intertidal and subtidal habitats in Monterey Bay. We investigated the absorption and assimilation efficiencies of these fishes to determine whether deep-sea species have evolved mechanisms to increase their efficiency of food use. Fishes were placed in experimental chambers and fed a known quantity of food. Ammonia excretion was measured and feces were collected daily. Both food and feces were analyzed for water, protein, lipid and ash to determine specific absorption efficiencies. Absorption ranged from 87.7 to 92.4% and assimilation efficiencies from 84.0 to 86.5%. Meal sizes from 0.5 to 4.0% of body weight did not affect the results. No significant differences were found between deep-sea and shallow-water species fed single meals or fed ad libitum for 10 days. This suggests that the selective pressure to maximize absorption and assimilation is universal and is not affected by the productivity of the habitat occupied. However, the relative size of the intestine in the deep-sea species was significantly smaller suggesting that they had a lower metabolic cost to maintain their digestive apparatus. It could not be concluded whether this was the result of pressure to conserve energy or rather the tendency of the shallow-living species to ingest more refractory material (i.e. sediment, algae).

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Introduction

Deep-sea fishes live in an environment with a low food supply when compared to the majority of shallow-water environments. Zooplankton biomass declines nearly exponentially with depth, exhibiting nearly an order of magnitude difference between the surface and 1,000 m (Angel and Baker 1982). Benthic animal biomass shows a similar decline (Haedrich and Rowe 1977; Haedrich et al. 1980; Lampitt et al. 1986; Merrett and Haedrich 1997). The adaptations that deep-sea animals have evolved to increase their ability to detect sparsely distributed prey in a dark environment and to maximize the number of successful attacks are well known (Marshall 1954; Young 1983; Herring 2002; Robison 2004). Extremely large eyes and mouths, fangs, bioluminescent lures, and expandable stomachs to fit even the largest of prey items are all examples.

More difficult to measure but also important are the ways in which these animals absorb and assimilate their food and finally allocate it to growth, reproduction, and maintenance. Selection will affect the relative investments in these energetic pools because they affect both immediate survival and reproductive potential. It follows that animals would also be adapted to maximizing energy gain from their food through digestion, absorption, and assimilation. The rate at which an animal

obtains energy (J s^{-1}) is the product of its feeding rate (g s^{-1}) and the digestibility or assimilation (J g^{-1}) of the food (Sibly and Calow 1986). In the deep-sea, low food availability could result in either infrequent meals or simply in a low daily feeding rate despite the various adaptations to maximize it. An animal confronted with this situation could evolve to maximize its assimilation capabilities. To do this an animal could increase the efficiency with which it extracts energy (cost per unit of absorbed energy) and/or evolve processes to extract more energy and materials from a meal (increased absorption and assimilation efficiencies, Sibly and Calow 1986; Penry and Jumars 1990).

This aspect of deep-sea animal energetics is relatively unstudied. The calculation of absorption (food energy – fecal losses) and assimilation efficiencies (absorption – excretory losses) requires controlled laboratory experiments in which both the animal's food and excretory products can be quantified. The difficulty in keeping deep-sea fishes and other animals alive in the laboratory has been a major obstacle to these types of studies. Nevertheless, by analyzing the caloric density of ingested prey and the rectal contents, Robison and Bailey (1981) were able to calculate relative absorption efficiencies of several midwater fishes. They showed that the efficiencies were higher for species with diets of gelatinous organisms and for deeper-living species that did not feed at night in the food-rich upper layers, suggesting that food availability was having an effect on digestive energetics. Hargrave et al. (1995) attempted to measure in situ absorption of food in the scavenging abyssal amphipod *Eurythenes gryllus*. Digestion proceeded rapidly but the inability to collect feces prevented the determination of absolute absorption efficiencies.

There are also strong arguments for a lack of any specific digestive adaptations in deep-sea animals. Studies have shown that depth-related declines of metabolic rates in some taxa are related to declining light levels and thus in the reactive distances between predator and prey, which reduces the need for strong locomotory capacity (Childress 1995; Seibel and Drazen 2006). This hypothesis is supported by a lack of metabolic declines in taxa, which do not rely heavily on vision, such as chaetognaths and medusae. Depth related declines in metabolic rates of pelagic taxa are similar between oligotrophic and eutrophic regions of the oceans, further suggesting that light levels rather than food availability drives the energetic adaptations of these animals. Certainly there is strong pressure in all environments to conserve energy if its expenditure is not needed. Thus the selective pressures for maximizing assimilation efficiency are likely to be the same across all environments (Pandian and Vivekanandan 1985). An investigation of assimilation efficiencies in deep-sea animals could provide new insights into whether they possess digestive adaptations to their environment.

Here we report results for two deep-sea, mesopelagic zoarcids which were carefully collected using remotely operated vehicles and maintained in the laboratory for extended periods of time. For comparative purposes we also examined the absorption and assimilation of a shallow-water zoarcid and an ecologically and morphologically similar stichaeid fish.

Methods

Four species of fish were used in this study, one stichaeid and three zoarcids. The stichaeid *Xiphister atropurpureus* lives from the intertidal to the shallow subtidal. *Eucryphycus californicus*, is a zoarcid primarily found at relatively shallow depths between 100 and 200 m (Cailliet and Lea 1977). *Melanostigma pammelas* and *Lycodapus mandibularis* are deep-sea pelagic zoarcids with most of their populations inhabiting depths between 400 and 1,200 m (Anderson 1980; Lancraft 1982; MBARI, VARS database). *Lycodapus mandibularis* migrates vertically on a diel basis and has been found within the top 100 m of the water column at night but mostly at depths >200 m (Anderson 1980). All of these fishes are of similar size and have similar diets consisting primarily of small crustaceans, although the diet of *X. atropurpureus* also includes some annelids (Kliever 1976; Anderson 1980; Barton 1982; Lancraft 1982; German and Horn 2006).

Prior to an experiment each fish was placed in a 7.5 l funneled aquarium and allowed to acclimate for several days. Each aquarium consisted of an acrylic cylinder 30 cm high and 20 cm in diameter. It was fused on the bottom to a plastic funnel. White plastic mesh (6 mm) was placed at the junction to keep the fish within the upper cylinder but also to allow the feces to sink into the funnel. The cap of a cryovial with its top removed was attached to the bottom of the funnel with a short piece of silicon tubing. This allowed us to quickly and easily collect feces and change the vial without the loss of water or disturbance to the animal. The aquaria used in this study resemble those described elsewhere for complete feces retrieval (Cho et al. 1982).

Two types of experiments were performed. Most of the experiments examined the response of the fish to a single meal. However, to examine the influence of meal frequency, individuals of *M. pammelas* and *E. californicus*

were also fed a meal once every day for ten consecutive days. We refer to these experiments as "multiple-meal" experiments. In both cases, an experiment was begun with a period of starvation equal to their average evacuation time (~2–3 days for shallow-living species and ~10 days for the deep-living species) of a single meal. Experiments were conducted at the normal environmental temperature for each species: *X. atropurpureus*, 15°C; *E. californicus*, 8°C; *M. pammelas* and *L. mandibularis*, 5°C.

For single meal experiments, each fish was fed a pre-weighed (±0.1 mg) meal of the krill, *Euphausia pacifica*, that ranged in size from ~0.5 to 4% of body weight. For multiple meal experiments, the same feeding procedure was followed every day. In some cases the fish did not feed every day or only a small amount. In all cases the total amount of food consumed was recorded. The krill were all collected in Monterey Bay during the same net haul and frozen at −80°C. Their size and chemical composition (protein, lipid and ash-free dry weight AFDW, $n = 27$) were nearly identical and an average composition was assumed for those krill fed to the fish. The composition of the krill was 79.77% water, 16.22% AFDW, 4.01% ash, 8.02% protein, and 1.23% lipid. The fish were accustomed to being fed by forceps so that typically, all of the food offered was consumed. Twelve hours after each feeding event the aquaria were inspected for pieces spat out or otherwise not consumed. The weight of these pieces was typically <5% of the total meal and it was subtracted from the weight of the meal. In a few instances this constituted ~10% of the meal, and the data for those experiments was not included in the analyses.

Feces produced by the fish passed through the mesh and settled to the bottom of each funnel and into the cryovial. The cryovials were replaced once or twice daily and the feces were removed to reduce the influence of leaching. Recent studies have indicated that leaching typically occurs only when feces are broken apart (Vandenberg and De La nouee 2001). This was not an issue in our experiments using single fish and gentle collection of individual fecal pellets. After collection, feces were blotted and weighed and their composition was determined. Inspection of the funnels continued for several days after the last feces were retrieved. This procedure ensured that complete meal evacuation had occurred.

Throughout both types of experiments, water samples were taken daily from the fishes' aquaria and from an identical control aquarium and were frozen for analysis of excretory products and the determination of excretory rates.

Nutrient-specific absorption efficiency was measured as the proportion of the meal (AFDW, lipid or protein) that was not lost as feces. In addition, lipid and protein were converted to energy equivalents (23.6 kJ g^{-1} protein, 36.2 kJ g^{-1} lipid, Brafield 1985) and total absorption was expressed as a percent of the total kilo joule consumed. Assimilation efficiency was measured as the proportion of the meal that was not lost as feces or urinary excretory products, in terms of energy equivalents. Resting excretory rates measured before feeding was used as a background rate. This rate was subtracted from the total over the duration of an experiment to determine excretion due to specific dynamic action. Ammonia and urea excreted were converted to energy equivalents (20.5 kJ g^{-1}; Brafield 1985). Ammonia concentrations were determined according to the method of Ivancic and Degobbis (1984), and urea assays followed Price and Harrison (1987).

Food (krill) and fecal samples were homogenized in distilled water and separate aliquots were used to determine protein, lipid, and ash in triplicate. The bicinchoninic acid (BCA) protein assay (Smith et al. 1985) was used with bovine serum albumin as a standard. Lipids were extracted according to Bligh and Dyer (1959) as modified by Reisenbichler and Bailey (1991) and quantified by sulfuric acid charring (Marsh and Weinstein 1966) using triolein as a standard. Water content was determined by the mass difference after drying to a constant weight at 60°C and ash was determined after combustion at 450°C for 24 h in a muffle furnace.

Comparisons of absorption and assimilation and intestine length to standard length (IL/SL) ratios between species were conducted using ANOVA. Unequal N Honestly Signficant Difference (HSD) post-hoc multiple comparisons were used. IL/mass ratios varied continuously with fish mass so ANCOVA was used for comparisons of the regressions. Standard *t*-tests were used for comparisons of fish fed single versus multiple meals. For some individuals, more than one experiment was conducted. Results for individuals were averaged before generating species-level data. For the analysis of the effect of meal size on absorption and assimilation, each experiment was considered an individual data point and regression analysis was used. All statistics were performed with the computer program Statistica (StatSoft, Inc. 2004. http://www.statsoft.com).

Results

Differences in gastrointestinal tract morphology were evident between species. The three zoarcids possessed two nublike pyloric caecae whereas *X. atropurpureus*

had 4–5 short but distinct caecae. *Melanostigma pammelas* and *L. mandibularis* have darkly pigmented peritoneums and stomachs, which is typical of deep-living fishes that consume and must conceal bioluminescing prey (McAllister 1961). The stomachs of the other two species are pale in color. The peritoneum in *E. californicus* is moderately pigmented but not black like the deep-sea species. *Melanostigma pammelas* and *L. mandibularis* have simple intestines. They pass posteriorly from the stomach then coil back to it before looping a second time toward the anus, producing a flattened "S" shape. The intestines of *X. atropurpureus* and *E. californicus* also pass posteriorly and then loop back toward the stomach. Then, in contrast to the deep-sea species, they have additional loops or twists before nearing the stomach and looping back to the anus. Thus there were significant differences in IL/SL between the species (ANOVA, $F_{3,42} = 49.802$, $P < 0.00001$). The two shallow-living species had significantly higher ratios than the deep-sea species (Fig. 1a; $P < 0.001$, Tukey HSD).

We also examined the relationship between IL and body mass. These regressions clearly showed a declining ratio with size in each species (Fig. 1b). Apart from

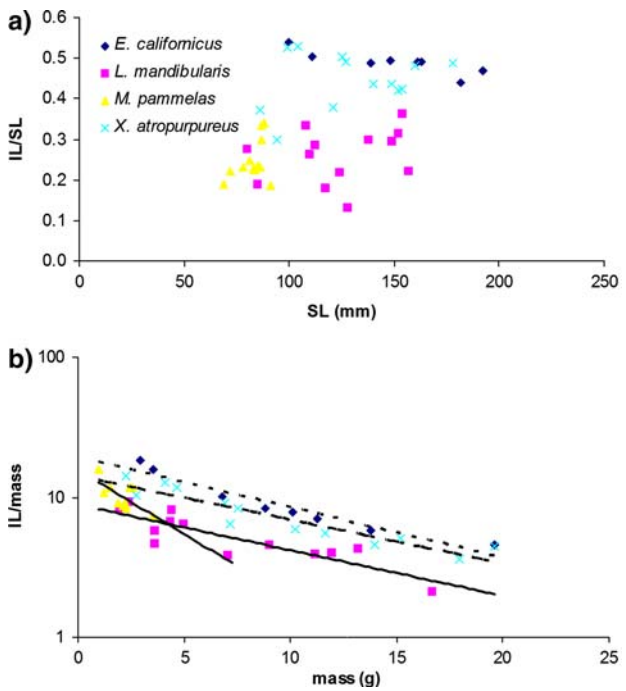

Fig. 1 The relationship between intestinal length and body size. **a** intestinal length as a proportion of standard length **b** intestinal length as a proportion of body mass versus body mass. Regression equations are *L. mandibularis*, $y = 8.86e^{-0.075x}$, $r^2 = 0.75$, *M. pammelas* $y = 15.73e^{-0.211x}$, $r^2 = 0.46$, *E. californicus* $y = 19.50e^{-0.083x}$, $r^2 = 0.93$, *X. atropurpureus* $y = 14.26e^{-0.072x}$, $r^2 = 0.88$. Symbols are as for *panel A*

M. pammelas, the species exhibited the same slopes (ANCOVA, $P > 0.05$). *Melanostigma pammelas* does not grow as large as the other species and the relationship between IL/body mass and body mass exhibited a steeper slope than the other species. The intercept for *L. mandibularis* was significantly lower than that for the two shallow-living species (ANCOVA, $P < 0.01$). In general the deeper-living species had lower IL/body mass ratios at all sizes than the shallow living species (Fig. 1b).

Also of interest, *X. atropurpureus* differed from all of the other species in that large specimens (>124 mm SL) possessed a rectum, a small section of the intestine distinctly larger in diameter than the rest, just prior to the anus. It averaged 15.8% of the entire intestinal length ($n = 8$). This is likely a vestige from herbivorous relatives in the group despite its carnivorous diet (Chan et al. 2004; German and Horn 2006).

Daily to twice daily feces collection allowed coarse measurement of total meal evacuation times. For *X. atropurpureus* and *E. californicus*, meals were evacuated in approximately 2 and 3.5 days, respectively. *Lycodapus mandibularis* evacuated meals in approximately 5.5 days and *M. pammelas* regularly took from 7 days to as much as 9.5 days to evacuate a single meal.

Total absorption ranged from 87.7 to 92.4% and assimilation efficiencies from 84.0 to 86.5% (Fig. 2). A significant difference was found between the species absorption efficiencies (ANOVA, $P > 0.05$) with *M. pammelas* having significantly lower absorption efficiency than the other three species (unequal *N* HSD, $P < 0.01$). There was no difference between the species' assimilation efficiencies (ANOVA, $P > 0.05$). On a component basis, the fish absorbed a substantial portion of the lipid, protein and AFDW of each meal (Fig. 3). *Melanostigma pammelas* absorbed slightly less protein than the other species (unequal *N* HSD, $P < 0.001$) and *E. californicus* absorbed less lipid than *X. atropurpureus* (unequal *N* HSD, $P < 0.025$).

The influence of both meal size and meal frequency (one meal versus daily feeding) were investigated. Meal sizes averaged 1.7–2.1% of body mass with a total range of 0.5–4.0%. Meal size showed no significant effect on any measure of absorption or assimilation ($P > 0.05$, Fig. 4). Only a few multiple-meal experiments were performed. Due to the lengthy food processing times for the deep-sea species these experiments were very long (up to 25 days). This complicated the measurements of excretion because of the need for water changes to prevent ammonia buildup and as a result, microbial degradation of ammonia occurred. Thus absorption data are available for these experiments but not assimilation data. The absorption data

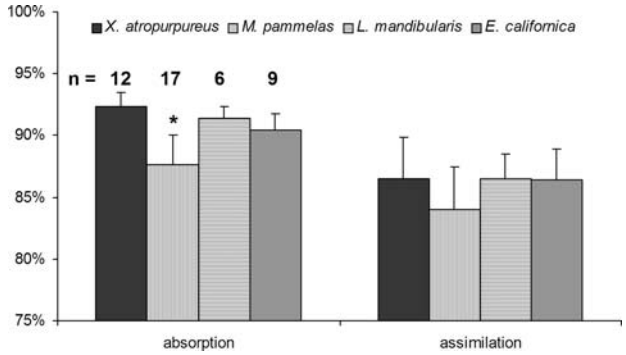

Fig. 2 Absorption and assimilation in the four fishes. Sample sizes for each species are given above the first bars. Error bars are standard deviation. * indicates a significant difference ($P < 0.05$)

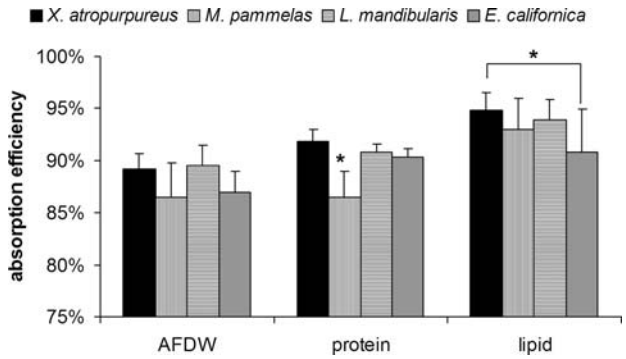

Fig. 3 Protein, lipid, and AFDW specific absorption in the four species. Sample sizes, *error bars*, and significance as in Fig. 2

suggest that the fish had slightly higher efficiencies when fed multiple meals (Fig. 5). This was primarily due to increases in protein absorption, the major constituent of the diet. Comparisons within species suggested that the slight elevation in protein absorption for *E. californicus* was significant (*t*-test, $P < 0.05$). There was also a significant reduction in lipid absorption with multiple meals in *M. pammelas*. As lipid content was relatively low in the krill fed to the fish throughout these experiments, this difference did not result in a significant difference in either absorption efficiency as measured by AFDW or in total energy.

Discussion

The possibility that a food-poor environment will lead deep-sea species to evolve the capacity to extract more energy and/or material from each meal is not supported by our results. All four species were very efficient at maximizing the energy gain from their food, with absorption efficiencies of 88–93% and assimilation efficiencies of ~85%. These results are similar to studies using similar methods of complete feces retrieval (Cho et al. 1982; Choubert 1999; Vandenberg and De La nouee 2001) and within the range of rates measured for carnivorous fishes (Brett and Groves 1979; Pandian and Vivekanandan 1985). There was little difference in digestive capabilities between shallow and deep-sea fishes fed single meals (Fig. 2). It would appear that these fishes are all adapted to make the most of the food they consume.

Digestion has inherent costs associated with the maintenance of the digestive tissues and with moving food through the gut. Thus, a species which can maintain assimilation at a high value but with a lower cost

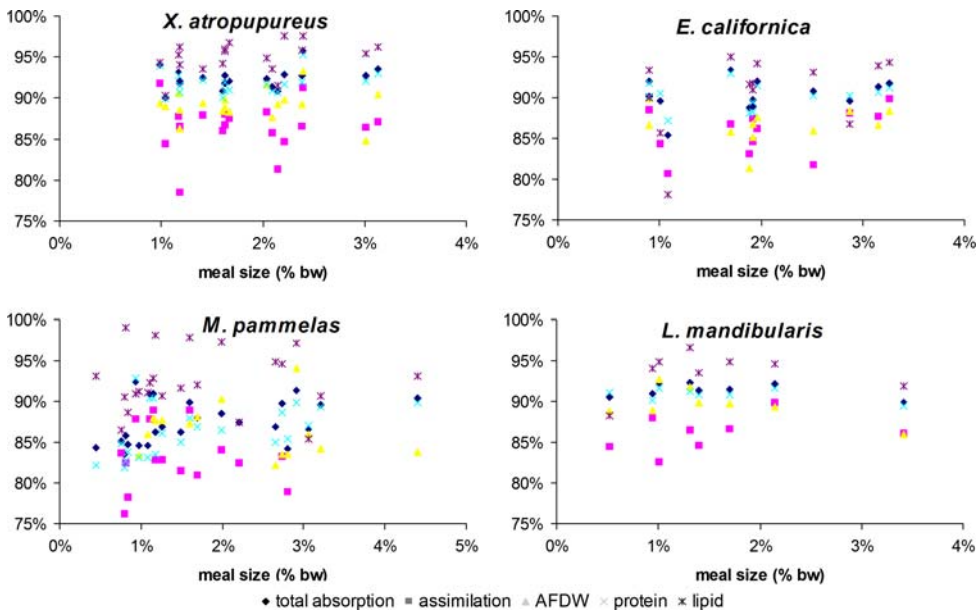

Fig. 4 The effect of meal size on absorption and assimilation efficiencies. For each fish if more than one experiment was conducted these data were plotted separately

could increase the efficiency of its energy use. If the costs associated with digestion are directly proportional to the length and mass of the gut, then longer guts will require greater metabolic maintenance costs and require more energy to move food a longer distance (Penry and Jumars 1987). Both deep-sea species in this study have shorter, less complicated guts than the shallow-living species, suggesting a lower cost for the process of digestion. In this respect they could be more efficient than the shallower-water fishes. Whether food availability drives this difference cannot be determined with the data at hand. Studies have shown that food type greatly influences gut length. Species consuming food of poorer quality have longer guts and exhibit similar assimilation or absorption efficiencies as species with short guts eating rich food (Kapoor et al. 1975; Robison 1984; Horn 1989; Kramer and Bryant 1995; Eastman and DeVries 1997). In fact fishes that exhibit ontogenetic changes in diet from carnivory to herbivory, show increases in gut length (Benavides et al. 1994; Cleveland and Montgomery 2003; German and Horn 2006). However, food type did not vary across the species in this study and varies little in the wild. All of them feed on small crustaceans (Kliever 1976; Anderson 1980; Barton 1982; Lancraft 1982; German and Horn 2006). The two shallow-water species are both benthic whereas the deep-sea species are pelagic, although they return to the sediment to spawn (Ferry-Graham et al. 2007). This is a constraint imposed by the limited availability of deepwater species with which to work. Benthic species often exhibit a diverse diet that can include sediment and detrital material and for *X. atropurpureus* it may include a small amount of algal material (Barton 1982). As a result these species may have longer guts than pelagic species. *Xiphister atropurpureus* possesses a rectum for enhanced digestion of refractory material and has a large gut relative to other carnivorous stichaeids (German and Horn 2006). The apparently lower digestive maintenance costs of the deep-sea species, while significant, may not relate to any adaptation to their environment per se but rather to an absence of selective pressure for the ability to process more refractory material.

Considering the time and energetic investment in procuring a meal, all organisms gain an energetic advantage by maximizing assimilation (Pandian and Vivekanandan 1985). Perhaps the only instances where reduced assimilation of food occurs are at the extremes of food availability. In such an instance a fish may gain more energy by consuming another meal as opposed to spending the time to completely digest the previous one. In these cases, throughput times decline and incomplete digestion is likely to occur (Kapoor et al. 1975; Sibly and Calow 1986). Meal size and frequency can be varied to investigate this hypothesis (Fänge and Grove 1979; Jobling 1993). The lack of contrast between deep- and shallow-living species persisted regardless of meal size (Fig. 4) or meal frequency (Fig. 5). Certainly meals larger than the maximum offered in this study (3–4% bw) could occur in the wild. However, such meals are probably infrequent and their energetic consequences minimal. With regard to meal frequency, both species were fed ad libitum daily yet their assimilation was not greatly affected. *Eucryphycus californicus* could have been fed more with multiple feedings each day but this is doubtful for *M. pammelas*. There are some data to suggest that mesopelagic vertical migrators may have lower digestive efficiencies than the species investigated here because they feed on zooplankton in the relatively rich epipelagic zone. Migrating myctophids have short guts, lower relative absorption efficiencies (Robison and Bailey 1981), and lower digestive enzyme activities (Gutowska et al. 2004), which suggest a high throughput, low efficiency digestive system. Deep-sea scavengers sporadically encounter food at high densities and are adapted to gorging themselves. At least one such scavenger, an amphipod, has rapid digestion (Hargrave et al. 1995) but the absolute absorption efficiency of these animals remains unknown.

Debate has occurred as to whether food availability limits the energetics of deep-sea species. Most of this has focused on metabolic rates which are as much as an order of magnitude lower than in shallow-living fishes, squids, and pelagic crustaceans inhabiting similar temperatures (Childress 1995; Seibel and Drazen 2006). Earlier studies explained the slow rates of deep-sea

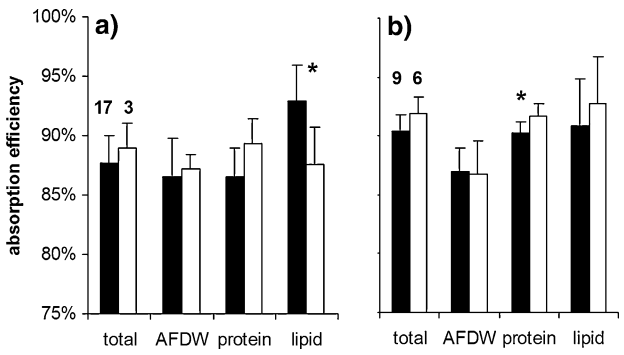

Fig. 5 Absorption of total energy, protein, lipid, and total AFDW in **a** *M. pammelas* and **b** *E. californicus* fed multiple meals compared to the data for single meals. *Black bars* represent data for fish fed single meals and are identical to those in Figs. 2 and 3. *White bars* represent data for fish fed multiple meals. Numbers above the bars for total absorption are sample sizes. *indicates a significant difference (*t*-test, $P < 0.05$)

animals as a limitation imposed by lower food availability at depth (Childress 1971; Smith and Hessler 1974). More recent studies have explained slower swimming speeds in deep-sea fish for the same reason (Collins et al. 1999; Priede et al. 2003). An alternative hypothesis for the observed decline in metabolic rates is that lower light levels relax the selective pressure for metabolic power (Childress 1995). This hypothesis, dubbed the "visual-interactions hypothesis" (Childress et al. 1990) suggests that in the absence of light and with low animal densities, the distances and frequencies with which predators and prey interact are reduced, resulting in a relaxed selection pressure for rapid locomotory capacity either to chase prey or evade predators. As a result, the need for a high metabolism no longer exists. Thus the selective pressure altering energetics is not in response to food supply. Instead the trends can be explained by a strong pressure in some shallow-water fishes and squids for rapid locomotion in clear sunlit surface waters. In a similar fashion, we suggest that low food availability itself does not provide a selective pressure for increased digestive efficiency in deep-sea animals. No differences in digestive capabilities were seen in the fishes we examined because all animals are selected for energetic economy, regardless of environment. We suspect that the generality of these results will be confirmed by testing on a more diverse array of species.

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