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Digestive chitinolytic activity in marine fishes of Monterey Bay, California

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Abstract

Chitinolytic activities, both chitinase (EC 3.2.1.14) and minimum chitobiase (β-*N*-acetyl-D-glucosaminidase; EC 3.2.1.30), were measured in stomach and intestinal tissues and their contents, from 13 fish species. Higher activities were found in the tissues than in the gut contents, and higher activities were seen in the stomachs than in the intestines. Demersal species exhibited chitobiase activities very close to their chitinase activities, suggesting that these fishes can degrade chitin completely to its soluble, absorbable monomer, *N*-acetyl-glucosamine. This suggests that these species may catabolize chitin not just to penetrate prey exoskeletons but also to derive nutrients from the chitin itself. In contrast, three mesopelagic species exhibited low chitobiase but high chitinase activities. This chitobiase limitation correlated strongly with gastrointestinal tract morphology, with the myctophids having the greatest chitobiase limitation and the shortest alimentary tracts. The high chitinase activities measured in the myctophids reflect their ability to rapidly disrupt prey exoskeletons ingested during their nightly feeding in surface waters. Their chitobiase activities are greatly reduced because with rapid meal evacuation through a short gut there is little time for processing and limited energetic advantage in the complete degradation of chitin. These results suggest multiple roles for chitinolytic enzymes in marine fishes and that feeding habits and frequency may have a bearing on the evolution of their digestive enzymes systems.

Keywords: Chitinase; Chitobiase; Chitin; Enzymes; Digestion; Diet; Marine fishes; Myctophidae; Nutrition

1. Introduction

Chitin is one of the most abundant carbohydrates present in the marine environment. Annual biosynthesis estimates range from 10^{10} to 10^{11} metric tons (Gooday, 1990), with estimates of 1–10 million metric tons of chitin in annual molts of the euphausiid *Euphausia pacifica* (Goodrich and Morita, 1977a), and amphipod inputs of as much as 3.7 g m^{-2} $year^{-1}$ in some estuaries (Gooday, 1990). Crustacean exoskeletons are the most common chitinous structure, but chitin is also synthesized by many other invertebrates, bacteria and algae. Chitin has a potentially important role in the ocean's food webs and carbon cycle because of its ubiquitous and extensive presence in marine ecosystems.

Chitin is a mucopolysaccharide polymer consisting of β-1,4-linked *N*-acetylglucosamine residues. Naturally occurring chitin varies in its degree of deacetylation and in its crystalline form (α,β,γ). In most organisms, chitin is found cross-linked with specific proteins and glucans to form structural units (Blackwell and Weih, 1984). Although most forms of chitin are resistant to chemical degradation, large areas of persistent chitin detritus have not been reported, and it is assumed that chitin is rapidly degraded by bacteria in the marine environment (Gooday, 1990; Lonhienne et al., 2001).

A system of exocellular enzymes typically carries out the biological decomposition of chitin. Chitinases (EC 3.2.1.14) and chitobiases (β-*N*-acetyl-D-glucosaminidase; EC 3.2.1.30) synergistically and consecutively hydrolyze the polysaccharide to monomers of *N*-acetyl-glucosamine (Jeuniaux, 1966). Chitinase hydrolyzes chitin chains into trimers and dimers while chitobiase further hydrolyzes the smaller units into *N*-acetyl-glucosamine monomers.

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Chitinolytic enzyme activities vary greatly between fish species and with the various methodologies used to examine them (Fänge et al., 1979; Lindsay, 1984). The primary function of chitinolytic enzymes is still debatable and likely varies between species. Along the alimentary tract of fishes, chitinolytic enzymes are believed to have various roles (Fänge and Grove, 1979; Clark et al., 1988; Jeuniaux, 1993). Chitinases are primarily associated with the stomach where they disrupt exoskeletons allowing other digestive enzymes access to nutrient-rich inner tissues. Chitinases have also been found in the intestines where they may aid in removal of fragment blockage (Lindsay, 1984). Chitobiases are mostly associated with the intestine, and pyloric caeca, where they further break down chitin into single units of *N*-acetyl-glucosamine (NAG) and may serve a nutritional function (Fänge and Grove, 1979; Clark et al., 1988; Jeuniaux, 1993). The enzymatic hydrolysis of chitin has the potential to result in additional energy gain from a meal.

In this study, chitinolytic activity was measured in stomach and intestinal tissues and contents of 13 fish species collected from Monterey Bay. Chitinase and chitobiase activities were measured separately to help elucidate the digestive and nutritional functions of chitinolytic systems in a diverse group of fishes. Our results provide insight into the ability of marine fishes to degrade chitin to an absorbable, nutritive monomer. The different chitinolytic systems identified here are discussed in relation to general feeding habits and feeding frequency.

2. Materials and methods

2.1. Sample collections

All samples were collected in Monterey Bay, California. Benthic fishes were collected by the National Marine Fisheries Service's (NMFS) Santa Cruz longline survey aboard the M/V Miss Alison. Mesopelagic myctophid fishes were captured in a 1.4 m^2 Tucker trawl aboard the R/V Western Flyer. *Pachycara* sp. and *Coryphaenoides armatus* were caught in baited traps deployed at 3500 m. All sample collections were made during the summer of 2002. Upon capture, samples were kept on ice while gastrointestinal tracts were quickly dissected out and frozen in liquid nitrogen. A few fish were kept on ice for a few hours before dissection and freezing, but this treatment should not have affected enzyme activity which is generally stable under a variety of storage conditions (Childress and Somero, 1979; Sullivan and Somero, 1980). *Pachycara* sp. and *C. armatus* were frozen whole at −40 °C for 2 days then transferred to −80 °C. Gastrointestinal (GI) tracts were dissected out of these latter fish while still partially frozen and were used immediately. Stomach and stomach contents, and intestine and intestinal contents were used from each fish, although some GI tracts had no contents. Stomach and intestinal fullness were noted as well as the type of food that had been consumed and its state of digestion. Special attention was paid to the presence of chitinous prey in the diet. pH measurements were taken of all the sampled GI tract contents. All sampled tissues were thoroughly rinsed with deionized water to remove any adhering contents and were carefully blotted. The standard and total length of each fish was measured, as was intestinal length.

Due to the small size of their GI tracts, material from four to five myctophids of the same species was pooled into one sample for measuring enzyme activities. In addition, complete separation of intestinal contents from the intestines was not possible in the myctophid samples, and the term "intestinal activity" refers to the activity of both the intestine tissue and the intestinal contents.

2.2. Homogenate preparations and enzyme assays

Tissue and content samples were homogenized in a glass homogenizer with a motor-driven pestle, using 0.15 M citric acid, 0.3 M Na_2HPO_4 buffer. Stomach and stomach contents samples were adjusted to pH 5, and intestine and intestinal contents samples to pH 7. Homogenates were then centrifuged for 5 min at 5000×*g*. The pH of the buffers were chosen to match normal GI tract values as reported in the literature (Bond, 1996) and our own simple pH measurements of the contents. Contents were often very mucous rather than liquid so pH was measured using pH paper (accuracy ±0.5 units).

Chitinolytic activities were determined using a modification of the standard procedure of Jeuniaux (1966) which measures the production of NAG. From each homogenate, a blank aliquot and two assay aliquots were taken. Each assay solution contained 1.0 mL of supernatant from the centrifuged homogenate and 0.5 mL of chitin solution. One assay aliquot contained 0.5 mL of β-glucosidase solution and the other contained 0.5 mL of distilled water.

The NAG concentration of the blank aliquot was determined in duplicate using the method of Reissig et al. (1955) modified for use on a microplate spectrophotometer after it had been centrifuged for 30 min at approximately 13,000×*g*. The concentration of NAG was determined by comparing each sample's absorbance at 585 nm to standard curves (0–280 μM NAG in both pH 5 and 7 buffers). Homogenate blanks were necessary because particle settling and the release of organic pigments were found to affect absorbance readings. Homogenate blanks also provided a blank for any NAG already present in the sample.

Test tubes containing the chitinase assay solutions were sealed and placed on a rotary shaker for 2 h at 20 °C. Afterwards, the entire volume of the chitinase assay was boiled for 10 min to stop the reaction and was centrifuged for 30 min at approximately 13,000×*g*. The NAG assay was performed as for the homogenate blank.

Color development in the NAG assay is very sensitive to pH, requiring pH 9.0 for adequate color development (Reissig et al., 1955). Because we used homogenization

buffers of different pHs for stomach and intestinal tissues, we had to use different reaction buffers to maintain the appropriate pH for color development for the NAG assay. Each NAG assay solution contained 0.5 mL of supernatant from the chitinase assay solution, 0.1 mL of $K_2B_4O_7$ buffer and 3.0 mL of *p*-dimethylaminobenzaldehyde (DMAB) solution. Samples which were homogenized in pH 5 buffer were buffered with 0.8 M $K_2B_4O_7$ adjusted to pH 10.8 with KOH. Samples homogenized in pH 7 buffer were buffered with 0.8 M $K_2B_4O_7$ adjusted to pH 9.3 with HCl. DMAB solution was prepared directly before use by adding 1.5 g of DMAB to 100 mL of glacial acetic acid containing 1.25% (v/v) HCl 12N.

For the chitinase assays, a chitin suspension of 5 mg/mL was prepared with purified chitin from crab shells suitable for analysis of chitinolytic enzymes (Sigma #C-9752). The solution was continuously mixed on a magnetic stir plate during pipetting to maintain a uniform colloidal suspension. A variety of synthetic chitin substrates have been used in past studies including glycol chitosan, chitin azure, and DNP-$(NAG)_4$ (Danulat and Kausch, 1984). However, it has been shown that synthetic substrates have different pH optima than a colloidal suspension of native chitin when digested with chitinolytic enzymes from crude biological homogenates (Clark et al., 1988). Enzyme activities measured with synthetic substrates can provide a relative index but the variability in their optima suggests that other enzymes are also involved in their cleavage, thus activity calculations cannot be purely attributed to chitinase activity. In this study, a colloidal suspension of native chitin was used as the substrate during the chitinase assay to limit the measurement of nonspecific lysozyme activity and to maintain the results in an ecologically relevant context.

The β-glucosidase (Sigma #G4511) solution had an activity of 6 units/mL. β-Glucosidase, which simulates the activity of chitobiose (Jeuniaux, 1966, 1993), was added to one of the two assays for each homogenate to allow for the measurement of total chitinolytic activity when chitin hydrolysis to NAG was limited by low chitobiase activity. Chitinolytic activities measured with the addition of β-glucosidase reflect total chitinase activity in the sample (Jeuniaux, 1966). When a large difference in chitinolytic activity occurred between the standard assay and β-glucosidase treatment, the activity of the nontreated sample can be inferred to reflect chitobiase activity. However, when the two treatments had similar activity, the nontreated sample cannot be inferred to represent chitobiase activity because chitinase activity could be rate limiting. Chitobiase limitation was measured by calculating the percent change in chitinolytic activity between β-glucosidase-treated and nontreated tissue incubations. All activities are expressed as μg of NAG produced per gram tissue (wet mass) per hour.

3. Results

Specimens of 13 fish species that commonly occur in Monterey Bay were sampled from depths of 100 to 3500 m in this study. In total, 3 of the species were mesopelagic myctophids and 10 species were demersal (Fig. 1).

Generally higher chitinolytic activity was found in the stomach tissue than in the stomach contents but many of the

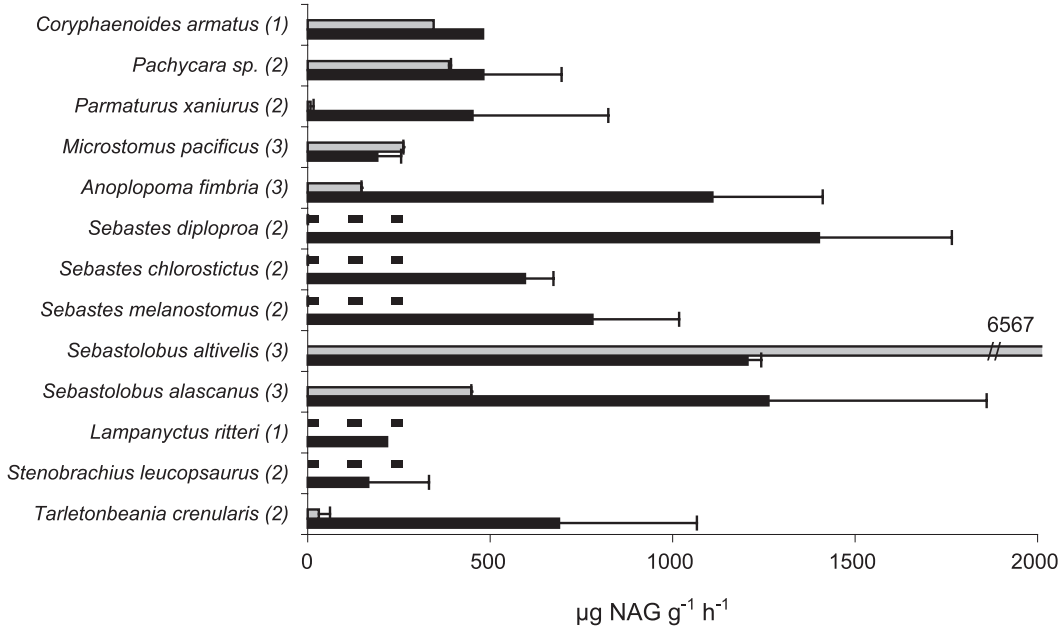

Fig. 1. Average chitinolytic activities measured in stomach tissue ■ and stomach contents □ (when available). The dotted bars indicate no data. Error bars represent standard error. Sample sizes are given in parentheses after each species name. For the myctophids, each sample represents pooled tissue from four to five individuals.

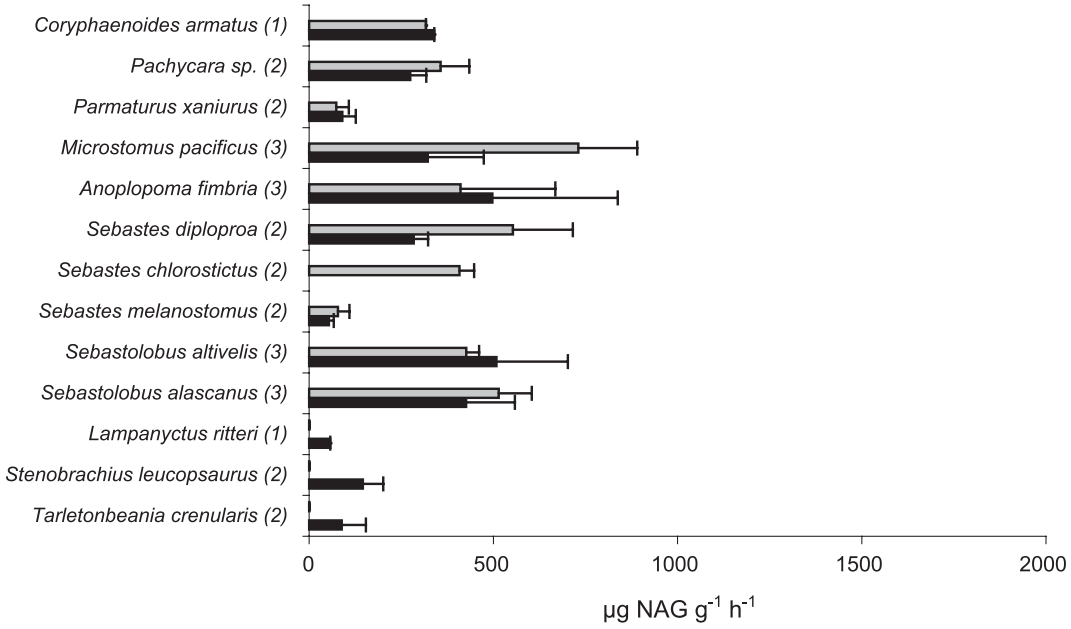

Fig. 2. Average chitinolytic activities measured in intestinal tissue ■ and intestinal contents □ (when available). Samples sizes and error bars are as in Fig. 1. For the three myctophid species, each bar represents the pooled activities for intestinal tissue and contents.

sampled fish had empty stomachs, so fewer stomach content measurements were available for comparison. The highest average chitinolytic activity was measured in the stomach contents of *Sebastolobus altivelis*. High activities were also measured in the stomachs of *Sebastolobus alascanus*, *Sebastes diploproa*, and *Anoplopoma fimbria*.

Chitinolytic activities were lower in the intestines and intestinal contents than in the stomach samples (paired *t*-test, $p<0.001$). Still, activity was present in all species (Fig. 2). Chitinolytic activities for intestinal tissue and contents were particularly high in *S. alascanus*, *S. altivelis*, *S. diploproa*, and *A. fimbria*, and also *Microstomus pacificus*.

High intraspecies variation was measured in all species (see error bars in Figs. 1 and 2). The presence of ingested chitin and gastrointestinal tract fullness were investigated as two possible sources of this variability. A correlation with digestive tract contents was confounded because all the sampled fishes had eaten chitinous material and the majority of the stomach contents were highly digested. There was also no relationship between stomach fullness and chitino-

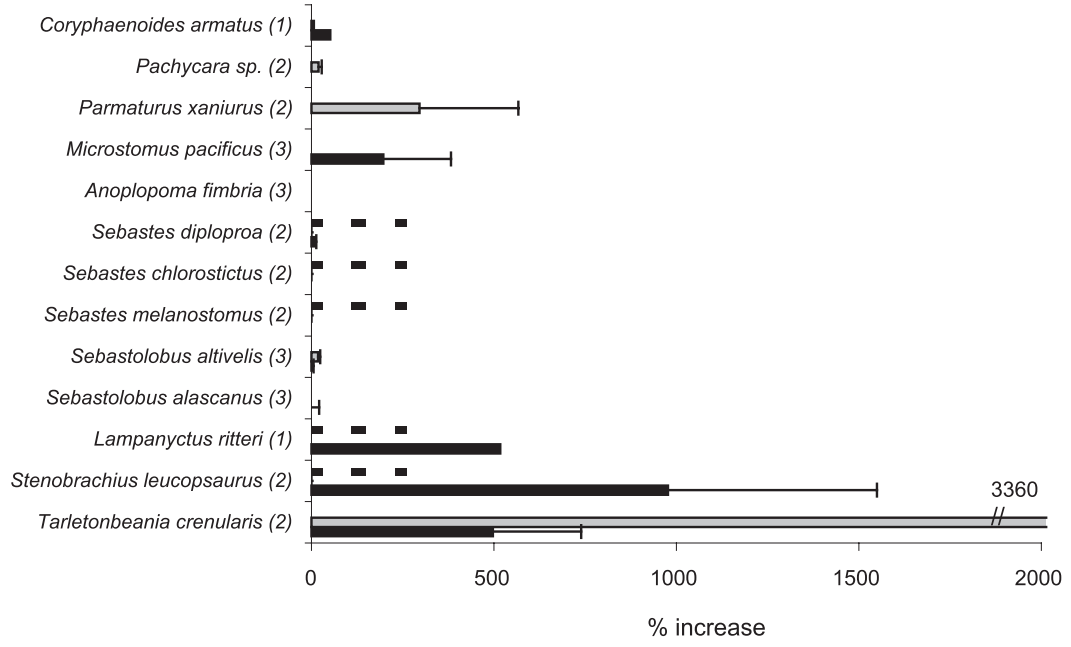

Fig. 3. Percent increase of chitinolytic activity in stomach tissue ■ and stomach contents □ (when available) with the addition of β-glucosidase. Error bars and sample sizes are as in Fig. 1.

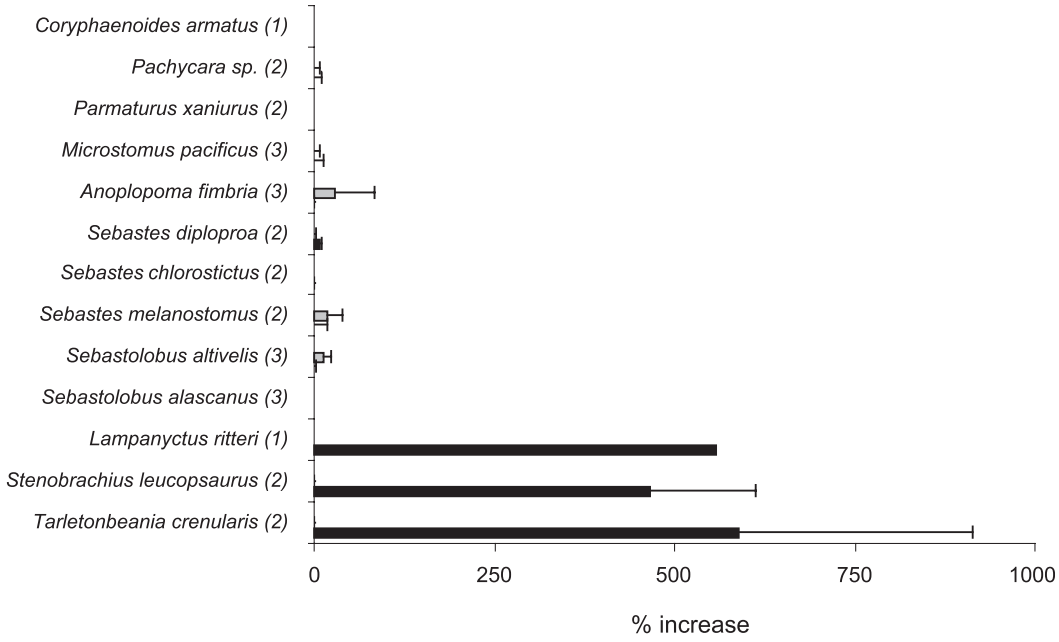

Fig. 4. Percent increase of chitinolytic activity in intestinal tissue ■ and intestinal contents □ (when available) with the addition of β-glucosidase. Error bars and sample sizes are as in Fig. 1. For the three myctophid species, each bar represents the combined activities for intestinal tissue and contents.

lytic activity for the four species in which there were both empty and full stomachs (paired *t*-test, $p>0.05$). For example, of the three *A. fimbria* specimens the highest activity was measured in a fish with an empty digestive tract and a specimen whose gut was filled with fish and squid was three times lower. All fishes had intestinal contents.

The data presented in Figs. 1 and 2 were calculated from assays that did not have β-glucosidase added to the initial incubations. Assuming the reactions were not rate-limited by chitinase activity, the data in the first two figures are indicative of chitobiase activity in the samples. To measure chitinase activity and illustrate possible chitobiase limitation, β-glucosidase was added to duplicate homogenate incubations. The majority of sampled species did not exhibit a large change in chitinolytic activity with the addition of β-glucosidase. Among the stomach samples (Fig. 3) and intestinal samples (Fig. 4), increases in chitinolytic activity were typically small or nonexistent. However, the exceptions included an approximate 3-fold increase in the activities of stomach contents from *Parmaturus xaniurus* and an approximate 2-fold increase in the activity of the stomach tissue from *M. pacificus*. Although there were large increases in activity, the total resulting chitinase activities were some of the lowest for all the fishes examined at 290 and 410 μg NAG g^{-1} h^{-1} for *P. xaniurus* stomach contents and *M. pacificus* stomach tissue, respectively.

Most dramatically, the three myctophid species exhibited very large and significant increases in chitinolytic activity of

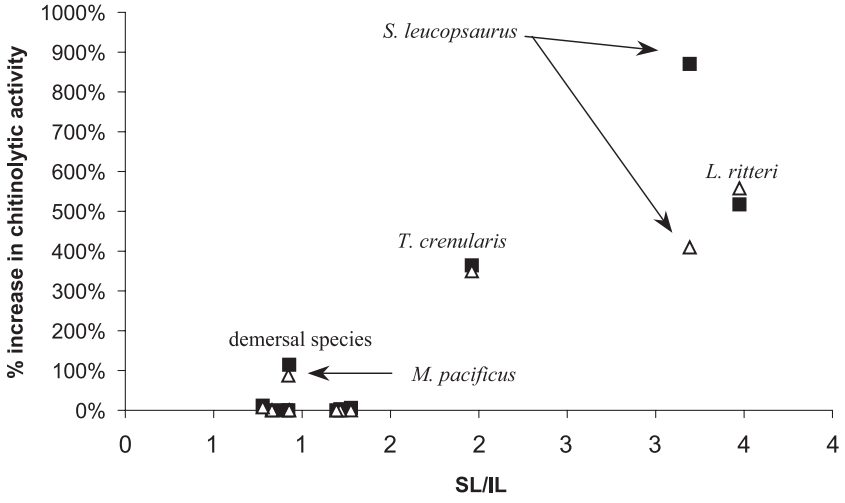

Fig. 5. The percent increase in chitinolytic activity in stomach (■) and intestinal (△) tissues after the addition of β-glucosidase versus the relative intestinal length (standard length/intestinal length) for each species. Demersal species includes all of the other species examined except for *C. armatus* and *P. xaniurus*.

both stomach tissue and contents (paired t-test, $p<0.05$). *Tarletonbeania crenularis* stomach contents exhibited the greatest increase, ~30-fold, among all the sampled fishes. *Stenobrachius leucopsaurus* had the largest increase for stomach tissue when incubated with β-glucosidase. At 980%, this increase was approximately twice as great as the values for both *T. crenularis* and *Lampanyctus ritteri*. These increases reveal that the myctophids have some of the highest chitinase activities of any of the fishes examined: *T. crenularis*, 3197 μg NAG g^{-1} h^{-1}; *S. leucopsaurus*, 1612 μg NAG g^{-1} h^{-1}; and *L. ritteri*, 1344 μg NAG g^{-1} h^{-1}. These results were paralleled in the intestinal tissues of these three species, with increases in chitinolytic activity of up to 600% (Fig. 4).

A correlation between chitinolytic activity and digestive morphology was apparent in the myctophid fishes (Fig. 5). As stated above, these species exhibited the greatest increases in chitinolytic activity with the addition of β-glucosidase. These three species also had the shortest intestines, reflected in a high ratio of standard length to intestinal length.

4. Discussion

Chitinolytic enzymes in the digestive systems of marine fishes can come from the fish, its prey, and the enteric bacteria. However, enzyme activities measured in the stomach, intestine, blood, liver and lymphomyeloid tissues have all been determined to reflect endogenous enzyme capabilities of fishes (Okutani, 1966; Fänge et al., 1979; Lundblad et al., 1979; Danulat and Kausch, 1984; Lindsay and Gooday, 1985; Danulat, 1986; Lindsay, 1986; Rehbein et al., 1986; Jeuniaux, 1993), and teleost chitinase genes have been found (Kurokawa et al., 2004) leaving little doubt that the majority of chitinolytic activity in their digestive tracts are produced by stomach and intestinal mucosa. However, this does not preclude the presence of extracellular bacterial chitinases, or the occurrence of interesting symbiotic relationships. In this study, chitinolytic activities measured directly from fish tissues are considered to reflect the enzyme producing capabilities of the fishes. Almost all of the fishes examined had higher activities in their tissues than in their stomach or intestinal contents, strongly suggesting that the measured enzyme activity is derived from the secretory glands of the fishes (Fänge et al., 1979). Certainly, we cannot preclude the presence of some bacterial chitinolytic activity in these samples without appropriate antibiotic treatment. Nevertheless, our estimates of activity reflect the natural conditions experienced by the fish and they are most relevant for analyzing digestive processes.

Chitinolytic activity was found in all of the fishes that we examined. While variation in techniques makes comparisons to other studies difficult, our measured activities are among the highest reported in marine fishes. Many of the species we examined had chitinolytic activities of up to 1500 (Fig. 1) and chitinase activities of up to 3000 μg NAG g^{-1} h^{-1} (Fig. 3) in stomach tissues. Fänge et al. (1979) reported chitinase activities of 1500–2100 in the gastric mucosa of the shark *Etmopterus spinax*, and Rehbein et al. (1986) reported gastric chitinolytic activities up to 2200 μg NAG g^{-1} h^{-1} for several species of Antarctic nototheniods. Many of the fishes we examined had intestinal tissue activities of between 250 and 500 μg NAG g^{-1} h^{-1} which is about 1.5 to 2 times higher than other studies using similar methodology (Fänge et al., 1979; Danulat and Kausch, 1984; Rehbein et al., 1986). It is probable that our optimization of the procedure of Reissig et al. (1955) for varying pH buffers is responsible for these differences.

The variability in activity was high both between species and within a species (Figs. 1 and 2), but such variability is common (Lindsay, 1984, 1987; Rehbein et al., 1986). Both the presence of chitin in the stomach contents (Goodrich and Morita, 1977b) and variation in feeding habits could affect the enzyme activities we measured. In this study, a correlation between chitinolytic activity and the presence of ingested chitin was not possible because all stomach contents contained chitin. General diet information is available for these species. The demersal fishes we examined have a wide range of chitinolytic activities but all are generalist feeders consuming a variety of squid, crustaceans, and fishes (Pearcy and Ambler, 1974; Buckley et al., 1999). Variation in diet would not seem to explain the variation in chitinolytic abilities. The single exception is the flatfish *M. pacificus* which feeds primarily on polychaete worms and ophuiroids (Gabriel and Pearcy, 1981). Its chitinolytic activities are among the lowest, which may reflect the lack of crustaceans in its diet. However, other species such as *P. xaniurus*, *Pachycara* sp., and *C. armatus* regularly consume crustaceans and have comparable low activities. The absence of a correlation with general feeding habits and chitinolytic activity has been noted before (Lindsay, 1984; Gooday, 1990). It has been shown that fish which swallow prey whole (such as the fishes in this study) have higher activities than those that mechanically disrupt their prey with pharyngeal teeth or by picking pieces of their prey (Lindsay, 1984).

The role that chitinolytic enzymes play in digestion can be elucidated in part by examining where they occur in the digestive tract and by examining both chitinase and chitobiase activity. Our results show chitinolytic activity in the stomachs that is 2–3 times higher than in the intestines (Figs. 1 and 2), a situation that is typical in fishes (Lindsay, 1984; Seiderer et al., 1987; Jeuniaux, 1993). These results and the acidic pH optima for chitinases have led to the conclusion that the chitinolytic enzymes in fishes are for the purpose of prey disruption and mechanical breakdown prior to passage through the intestine. Chitinases hydrolyze the chitin polysaccharides into insoluble dimers or trimers, so the physical disruption of prey exoskeletons is a logical role (Jeuniaux, 1966; Fänge and Grove, 1979; Gooday, 1990). This action facilitates the penetration of other digestive

enzymes into prey tissues (Lindsay, 1984; Seiderer et al., 1987; Clark et al., 1988) and it is particularly important because the majority of fishes do not masticate their prey (Bond, 1996). However, the function of these enzymes may be more complex. High intestinal activities have been measured in this study (Figs. 1 and 2) and others (Danulat and Kausch, 1984; Rehbein et al., 1986; Seiderer et al., 1987; Clark et al., 1988). Lindsay (1984) has suggested that chitinase in the intestine may act to degrade chitin particles or masses that might obstruct flow in the intestine.

Chitinolytic enzymes are probably used for more than prey disruption. We found chitobiase activities as high as the chitinase activities. Chitobiase cleaves the NAG dimers into soluble, individual units of *N*-acetyl-glucosamine (Jeuniaux, 1966; Fänge and Grove, 1979; Gooday, 1990). Using the standard procedure of Jeuniaux (1966) to measure chitinolytic activity is really a measure of chitobiase activity (the last step of the reaction) unless a supplement of β-glucosidase is added. If assays are run both with and without the supplement, the increase in activity upon this addition is a measure of the chitobiase limitation of the system and the supplemented activity is the true chitinase activity. Except for two demersal species and the three myctophids that are discussed below, our results gave little evidence of chitobiase limitation (Figs. 3 and 4), suggesting that chitinases and chitobiases were present with nearly equivalent activities. It must be pointed out that where little or no chitobiase limitation was observed, the estimates of chitobiase activity should be considered minimums because the chitinolytic system in the tissues examined could have been chitinase limited.

It seems unlikely that fishes would produce significant amounts of chitobiases if the sole function of the chitinolytic system was the disruption of prey exoskeletons. Our results support the idea that chitinolytic enzymes in fishes have multiple digestive functions. Chitinases perform a digestive function when they disrupt prey exoskeletons in the stomach and prevent fragment blockages in the intestines. Chitobiases perform a nutritive function when they complete chitin degradation by breaking down chitin dimers into absorbable nutritive monomers of NAG. Despite past opinions (Jeuniaux, 1993; Jobling, 1993), our data and that of other recent investigations (Matsumiya and Mochizuki, 1996; Place, 1996) suggest that the latter function is important in teleosts. The nutritive value of NAG has been examined in several studies. NAG absorption has been documented in several fish species (Peres et al., 1973) and more specifically, the intestinal mucosa of *Scylliorhinus canicula* was found to absorb NAG more easily than glucose (Alliot, 1967). Kono et al. (1987) have also shown that adding chitin to the diet of fish can increase growth rates and assimilation efficiencies in aquaculture. In a survey of crustaceans off the California coast, Childress and Nygaard (1974) found that chitin constituted 2–16% of the ash-free dry weight of the animals. With a caloric value of ~17.1 kJ g^{-1} (similar to protein), chitin can represent a large fraction of the potential energy in these prey.

The three myctophid species that we examined were strikingly different from the demersal species and they provide a good example of how feeding habits may affect chitinolytic enzyme systems. In contrast to most of the demersal fishes, they exhibited strong chitobiase limitation (Figs. 3 and 4). Demersal *M. pacificus* stomach tissue was also chitobiase limited but both its chitobiase and chitinase activities were very low, setting it apart from the myctophids. The stomach contents of *P. xaniurus* were also strongly chitobiase limited but its stomach tissue was not, suggesting that exoskeletal moulting chitinases from its last meal of shrimp contributed significantly to its activity. For the myctophids and perhaps *M. pacificus*, a much lower chitobiase activity compared to high chitinase activities suggests that these fishes use chitinolytic enzymes primarily to disrupt prey, conforming to the more conventional hypothesis for enzyme function (Lindsay, 1984; Seiderer et al., 1987). Their chitobiase activities are among the lowest in this study (Figs. 1 and 2), but their chitinolytic activity increased ~3.5 to 9 times upon addition of β-glucosidase, revealing some of the highest stomach tissue chitinase activities of any fish examined.

These three species are mesopelagic and migrate vertically at night to nutrient rich surface waters where they feed primarily on crustacean zooplankton (Tyler and Pearcy, 1975; Gartner et al., 1997). They have relatively small intestinal length to standard length ratios compared to the demersal fishes without chitobiase limitation (Fig. 5). With pronounced nightly feeding bouts in relatively food rich waters, these fish must evacuate their food in less than 24 h so that they are ready to feed again the following night (Gartner et al., 1997). Therefore, high chitinase activities probably allow them to quickly disrupt prey exoskeletons to facilitate other digestive enzymes access to tissues. With a brief residence time in a short gut, there may not be enough time to efficiently digest and absorb the chitin. Furthermore, with regular feeding bouts, the potential energetic gain from the chitin may be relatively small and not worth the production of the chitobiases.

This work demonstrates a correlation between gross anatomical features, feeding habits and chitinolytic enzyme capabilities. Future work needs to examine a broad diversity of fishes with varying feeding habits using comparable methodology. Investigations of the absorption of NAG and the assimilation of chitin in a variety of fishes are also required so that the entire spectrum of chitinolytic adaptation can be examined.

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