Changes in enzymatic activity during early development of bay scallops *Argopecten irradians* and sea scallops *Placopecten magellanicus*

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ABSTRACT: Poor growth and survival of scallops is often reported during early developmental stages. Stage- and species-specific dietary requirements have been identified, but the mechanisms responsible for these differences remain largely unknown. Dietary success depends on food biochemical properties, digestive and/or assimilative capacity, as well as the animal's specific nutritional demands, which can vary over development and between species. The present study examines developmental changes in the activities of key digestive enzymes in larval and postlarval bay scallops *Argopecten irradians* and sea scallops *Placopecten magellanicus* raised on a mixed algal diet until ~4 to 5 mm in shell height (SH) and sampled at intervals encompassing major transitions in feeding organ development. Colorimetric assays measured general protease, lipase (esterase) and carbohydrase (α -amylase, cellulase and laminarinase) activities. The most pronounced changes in enzymatic activity occurred prior to scallops attaining ~1.2 mm SH in both scallop species. The esterase:protease ratio exhibited similar ontogenetic patterns in both scallop species, decreasing sharply between larval and immediate postlarval stages before increasing to an intermediate level and stabilizing around 1.2 mm SH. In contrast, mean carbohydrase activities measured over the experimental duration differed between species, with bay scallops exhibiting higher specific activities of α -amylase and cellulase but much lower activities of laminarinase than sea scallops. These findings have implications for understanding scallop utilization of the food supply in nature, as well as developing targeted diets that could enhance their growth and survival in culture.

KEY WORDS: Digestive enzyme activity · Scallops · *Placopecten magellanicus* · *Argopecten irradians* · Development

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INTRODUCTION

Larval and early-postlarval development of bivalves is marked by pronounced changes in behavior, physiology, habitat and morphogenesis of feeding organs (Sastry 1965, Beninger et al. 1994, Kennedy 1996, Barré 2001, Chaparro et al. 2001, Veniot et al. 2003). Stage- and species-specific differences in dietary needs have been identified for different bivalve species, including the scallop *Argopecten purpuratus*

(Uriarte & Farias 1999 and references therein, Milke et al. 2006), although the mechanisms responsible for these requirements are not fully understood. While biochemical composition of the diet is undoubtedly important, the ability to digest and assimilate various substrates is also necessary to ensure optimal growth and survival. Digestive capacity may develop in parallel with morphogenesis of feeding organs, and the activities of various digestive enzymes may influence dietary success.

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Digestive capacity may also differ between species with differing life histories or environmental adaptations (Albentosa & Moyano 2009). The sea scallop *Placopecten magellanicus* is restricted to cold waters ($\leq 20^{\circ}\text{C}$; Naidu 1991) and growth rates are relatively slow, attaining a market size of 90 mm shell height (SH) in ~3 yr (Dadswell & Parsons 1991). The northern bay scallop *Argopecten irradians irradians* is a temperate subspecies with natural populations occurring in subtidal estuarine environments (Couturier et al. 1995). They are generally restricted to waters <10 m in depth, and grow much faster than sea scallops. Bay scallops are capable of attaining market size (50 mm SH) in a single growing season (Rhodes & Widman 1980).

A number of studies have determined the activity of digestive enzymes of adult bivalves including bay scallops (Brock et al. 1986) and sea scallops (Wojtowicz 1972), and a few studies have focused on the digestive enzymes of larval and juvenile bivalves (Saimain et al. 1995, Økland et al. 2001, Hoehne-Reitan et al. 2007, Albentosa & Moyano 2008). Prior studies examining ontogenetic changes in digestive enzymes have been conducted on abalone (Takami et al. 1998), teleosts (Gawlicka et al. 2000, Pérez-Casanova et al. 2004, Jun-sheng et al. 2006) and crustaceans (Kamarudin et al. 1994, Ribeiro & Jones 2000). Studies on ontogenetic digestive changes in bivalves, or that compare discrete life history stages, are limited to those conducted on *Crassostrea gigas* (Luna-González et al. 2004) and *Pecten maximus* (Økland et al. 2001, Reitan 2011).

The objectives of the present study were to (1) identify changes in digestive capacity measured by *in vitro* activity of digestive enzymes during early scallop development and (2) compare activities of key enzymes between *Argopecten irradians* and *Placopecten magellanicus*. This information could assist in the development of targeted diets for implementation in a commercial setting and may also increase understanding of bivalve–seston interactions in the natural environment.

MATERIALS AND METHODS

Experimental animals

Larval bay scallops *Argopecten irradians* were obtained from Lunenburg Shellfish Inc., Nova Scotia (NS), Canada, and transferred to the National Research Council's Marine Research Station (MRS), Ketch Harbour, NS, where they were held on a mixed diet (30 cells μl^{-1} ; 50%:50% by volume) of *Pavlova*

sp. (Center for the Culture of Marine Phytoplankton [CCMP] Strain 459)/*Chaetoceros muelleri* (CHGRA) at 20°C for 4 d prior to initial sampling at the pediveliger stage. Post-metamorphic bay scallops from the same cohort were received 10 d later and treated as above. Due to high mortalities after Day 11, the initial cohort of bay scallops (Cohort 1) was replaced on Day 19 by a second, 7 d younger cohort (Cohort 2, initial SH = 700 μm) originating from the same broodstock. No sampling was conducted during the period of mortality in Cohort 1, and Cohort 2 was acclimated to the experimental diet for 6 d prior to sampling. Sea scallop *Placopecten magellanicus* larvae were spawned and reared at MRS and held on a diet of *Pavlova pinguis*/CHGRA at 14°C for 3 d prior to initial sampling. At this point sea scallops were sieved into 3 different size classes. Larvae and the smallest early postlarvae (526 μm SH) were sampled, and remaining sea scallops were used for subsequent post-metamorphic samplings.

Experimental systems

Larval scallops were held in aerated, 0.22 μm filtered seawater on a mixed algal diet in 60 l (bay scallop) or 700 l (sea scallop) tanks prior to initial sampling; larvae were sampled only once. For postlarval experiments, approximately 200 000 bay scallops (initial SH = 310 μm) and 120 000 sea scallops (initial SH = 773 μm) were divided equally among 4 downwellers, 2 per each duplicate 400 l (bay scallops; 125 μm initial mesh size) or 700 l (sea scallops; 130 μm initial mesh size) recirculating seawater system. Downweller mesh sizes were increased commensurate with shell growth; bay and sea scallops reached a maximum of 1180 and 1000 μm SH, respectively. Each scallop species was grown at its optimum temperature, i.e. 20°C and 14°C for bay and sea scallops, respectively.

Microalgal stock cultures were obtained from the CCMP, West Boothbay Harbor, Maine, USA, and consisted of *Pavlova* sp. (CCMP 459; Pav 459), *Pavlova pinguis* (CCMP 609) and *Chaetoceros muelleri* (CCMP 1316; CHGRA). CHGRA and Pav 459 were grown in batch culture in 20 l carboys at 20°C , while *P. pinguis* was grown in semi-continuous culture in a 200 l photobioreactor at 21°C (the irradiance delivered to the culture volume inside the photobioreactor was $350 \mu\text{mol m}^{-2} \text{s}^{-1}$, as determined at the surface of the Pyrex sleeve surrounding each lamp). All species were grown with 24 h light, on *f/2* (without silica Si for flagellates; Guillard 1975) with supplemental CO_2 and were harvested during the late exponential growth phase.

Scallops were fed a binary diet of either Pav 459 or *Pavlova pinguis* in combination with CHGRA for 47 d (bay scallop) or 65 d (sea scallop) at $2.16 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$; equivalent in biovolume to 50 cells μl^{-1} of *Isochrysis galbana* Strain T-iso. A diet of Pav 459 in combination with CHGRA, at this concentration, has resulted in high growth rates in postlarval bay and sea scallops (Milke et al. 2004, 2006). *P. pinguis* and Pav 459 are comparable in size and biochemical composition and have supported comparable growth rates in postlarval sea scallops when used in combination with CHGRA (Milke et al. 2008), so it was assumed that they could be interchanged without effect. Each algal species contributed to the diet equally by volume, and cell concentrations were monitored with a Multisizer 3 Coulter Counter (Beckman Coulter). Diets were maintained within $\leq 20\%$ of the target concentration by daily batch additions and drip supplementation via peristaltic pump (Cole Parmer Masterflex 7554-80) when necessary.

Scallop growth and survival

Growth and survival was measured weekly via image analysis. Video of scallops from each downweller was taken using a Pulnix TMC7-DSP camera coupled with a dissecting scope (or lens for larger animals) and recorded on a Sony DVCAM (DSR-V10). Shell height determinations (umbo to distal shell edge for postlarvae; shell length was measured in larvae) were conducted by measuring 30 (bay scallop) or 50 (sea scallop) live scallops per downweller using Optimas 6.5 image analysis software (Media Cybernetics). For mortality estimates, in a sample 100 scallops per downweller were counted as live, 'new' dead and 'old' dead, converted to percent data, and then averaged to obtain tank and overall mortality for each sampling date. 'New' dead scallops were similar in size to live animals but showed no movement and/or partial tissue degradation, while 'old' dead scallops were smaller, they showed no movement but evidence of substantial or complete degradation of tissues.

Enzymatic assays

Seven to 9 samplings were conducted over time for analysis of enzyme activities up to a maximum size of 3.9 and 4.9 mm SH in bay and sea scallops, respectively. For each sampling, a subset of scallops was removed from each downweller and placed in filtered

seawater (0.22 or 1 μm) overnight to clear gut contents and to ensure that enzymatic measurements were not influenced by the presence of microalgae; preliminary experiments were unable to detect statistically significant effects of this brief starvation period on enzymatic activity ($p = 0.10, 0.75, 0.21$ and 0.20 for esterase, protease, α -amylase and laminarinase, respectively) except marginally for cellulase ($p = 0.046$). The number of scallops in each sample was recorded, pooled whole scallops (including shell) were placed into cryovials, and samples were frozen in liquid N_2 after removal of excess water. Samples were stored at -80°C until analysis. Two tanks were sampled at each size, and 2 downwellers per tank, which yielded 2 discrete enzyme samples per tank. Downweller values were averaged to provide 1 tank value; therefore, values are more robust than is indicated by our sampling size of $n = 2$ for statistical analysis.

All samples were lyophilized and subsequently homogenized using a Polytron homogenizer, or by hand using a Teflon-coated rod for smaller samples, in 100 mM ammonium bicarbonate buffer, pH 7.8 (1 g tissue wet weight:5 ml buffer) on ice in order to minimize proteolytic degradation. Samples were centrifuged at 1700 g at 4°C for 10 min, aliquoted into 0.5 ml centrifuge tubes and stored at -80°C . Before analyses, samples were thawed on ice and again centrifuged at 5100 g at 4°C for 2 min; only supernatant was used in assays. All protein concentration and enzymatic activities were determined colorimetrically on a SpectraMax Plus or VMax 96-well microplate reader (Molecular Devices) and analyzed using SoftMax Pro software Version 4.7.1 (Molecular Devices). Soluble protein was determined using a modified Lowry protocol (Bio-Rad; Lowry et al. 1951) using bovine serum albumin as standard and detection at 650 nm. Preliminary trials determined the optimum incubation time and pH for each enzyme assay; all analyses were conducted at 30°C , allowing for direct comparisons between different enzymes and scallop species. This temperature was chosen as it was within the range of temperature optima for carbohydrases in bay scallops (28 to 32°C ; Brock et al. 1986), and following work on *Placopecten magellanicus* digestive enzymes by Wojtowicz (1972), who used 37°C for laminarinase, 25°C for amylase and 30°C for most other carbohydrases. Maximum individual enzymatic activities in ectotherms are typically attained at higher temperatures than the organism's upper environmental tolerance range (Le Chevalier et al. 1995, Pörtner 2002). Specific activities of 5 different enzymes were determined in the present study.

Protease activity was determined using the azocasein hydrolysis assay (Charney & Tomarelli 1947) modified by Ross et al. (2000) for microplate analysis. Homogenate was incubated with azocasein (Sigma A2765; final concentration of 3.5 mg ml⁻¹ dissolved in 100 mM ammonium bicarbonate buffer; pH 7.8) for 19 h on a rotator at 60 rpm. The reaction was stopped with the addition of 20% trichloroacetic acid (final concentration of 4.5%); samples were cooled on ice and tubes were centrifuged at 17 000 *g* for 5 min, after which equal volumes of homogenate and 0.5 M NaOH were added to microplate wells. Optical density was measured at 450 nm, and 1 U of activity was defined as the change in optical density per hour.

For determination of esterase activity, the homogenate was incubated with 0.4 mM *p*-nitrophenyl myristate (Sigma; Huggins & Lapidés 1947) in 100 mM ammonium bicarbonate buffer (pH 7.8) with 0.5% Triton X-100. Change in optical density was monitored continuously for 1 h at 405 nm, and 1 U of activity was defined as 1 µmol of *p*-nitrophenol liberated min⁻¹ at 30°C. The extinction coefficient of *p*-nitrophenol in microplate wells was determined empirically.

A *p*-hydroxybenzoic acid hydrazide (PAHBAH)-reducing sugar assay was adopted for carbohydrase assays (Lever et al. 1973, Lever 1977), where an initial sample (allowing for no incubation time of substrate and tissue homogenate) was adopted as the sample blank and 1 mg ml⁻¹ D-glucose (AnalaR) was used as standard. Briefly, substrate, buffer, H₂O and tissue homogenate were added to 1.6 ml centrifuge tubes and incubated at 30°C for 30 min, after which the 4-hydroxybenzoic acid hydrazide (PAHBAH) reagent was added to stop the reaction. Samples were heated at 70°C for 10 min to facilitate color development. After cooling, samples were added to microplate wells, and optical density was read at 405 nm. Final substrate and buffer concentrations, prior to PAHBAH addition, were as follows: α-amylase, 0.5% soluble starch (Sigma, S-2630) and 0.08 M sodium citrate buffer pH 6.5; cellulase, 1% carboxymethyl cellulose (Sigma C-5678) and 0.05 M phosphate buffer pH 6.0; laminarinase, 0.2% laminarin (Sigma L-9634) and 0.08 M sodium citrate buffer pH 5.5. Substrate concentrations were based on previous studies of marine organisms (Gawlicka et al. 2000, Fernández-Reiriz et al. 2005). Due to the high activity of laminarinase in the samples, tissue homogenates were diluted 1:1 with buffer prior to incubation. The assay measured the release of glucose-reducing group equivalents, and 1 U of activity was defined as the release of 1 µmol of glucose min⁻¹ at 30°C.

Statistical analysis

Scallops were divided into 3 distinct morphological stages: (1) larvae, (2) early postlarvae <1 mm SH and (3) late postlarvae >1 mm SH. These were selected based on the known occurrence of major developmental changes in the functional morphology of the feeding organs, especially the gills, in the 2 scallop species (Barré 2001, Veniot et al. 2003). One-way ANOVAs were conducted to determine differences among these morphological stages for each scallop species and digestive enzyme. When significant differences were identified, pairwise multiple comparisons were conducted using the Holm-Sidak method (SigmaPlot 11.2, Systat Software). In the few cases when assumptions of normality and equal variance were not met, a Kruskal-Wallis 1-way ANOVA on ranks was conducted, followed by a Dunn's method pairwise multiple comparison test when appropriate. *T*-tests were conducted to examine species-specific differences for each digestive enzyme, combining all shell heights; a Mann-Whitney rank sum test was conducted in 2 cases when the assumptions of a *t*-test were not met. Analyses were conducted on SigmaPlot 11.0; the α-level was chosen as *p* < 0.05.

RESULTS

Scallop growth and mortality

Growth of scallops fed the *Pavlova* spp./CHGRA diets throughout the experiment (Fig. 1) was best described by exponential equations and yielded predicted mean daily growth rates of 157 and 107 µm d⁻¹ for bay and sea scallops, respectively. Total mortalities of bay scallop Cohort 1 were fairly low (~15%) during the first 11 d of the experiment, when sampling for enzymatic analysis was conducted. Scallops from Cohort 2 exhibited 76% survival by the end of the experiment at Day 47. Exceptionally low mortalities were observed during the sea scallop trial, with a final survivorship of 95% at Day 65.

Enzymatic activity during development

Soluble protein concentrations were variable over development, and ranged from 35.8 to 87.6 and 24.0 to 93.4 mg g⁻¹ DW for bay and sea scallops, respectively, with a reduction in values obtained immediately following metamorphosis in both species (Fig. 2). The specific activity of esterase was signifi-

Fig. 1. *Argopecten irradians*, *Placopecten magellanicus*. Post-metamorphic shell growth trajectories of bay scallops *A. irradians* and sea scallops *P. magellanicus*. Values represent mean shell height ($\pm$ SE); arrow indicates size at which the initial bay scallop cohort was replaced with a second batch of animals (see 'Materials and methods: Experimental animals')

Fig. 2. *Argopecten irradians*, *Placopecten magellanicus*. Soluble protein concentrations based on lyophilized weight of samples. Values represent means ($\pm$ data range); $n = 2$ per shell height

cantly reduced between larval and early postlarval stages in both scallop species (Fig. 3). It remained at similar levels between early and late postlarval bay scallops (0.37 and 0.36 U g^{-1} protein, respectively), but for sea scallops it exhibited a significant increase between early and late postlarval stages (0.27 to 0.45 U g^{-1} protein). The opposite effect was found for the specific activity of protease (azocasein): activity significantly increased between larvae and early postlarvae for both scallop species. It subsequently remained constant in sea scallops, but dropped sig-

Fig. 3. *Argopecten irradians*, *Placopecten magellanicus*. Specific activities of digestive enzymes among 3 discrete morphological stages: (1) larvae, (2) early postlarvae <1 mm shell height and (3) late postlarvae >1 mm shell height for bay scallops *A. irradians* (upper panels) and sea scallops *P. magellanicus* (lower panels). Specific activity was defined as the release of 1 μmol of p-nitrophenol $\text{min}^{-1} \text{g}^{-1}$ protein (esterase), the change in optical density per hour per milligram protein (protease) and the release of 1 μmol glucose $\text{min}^{-1} \text{g}^{-1}$ protein (α -amylase, cellulase, laminarinase). Values represent means ($\pm$ SE); different letters designate significant differences among developmental stages; 1-way ANOVA; $p < 0.05$. For bay scallops $n = 2, 6$ and 9 for larvae, early postlarvae and late postlarvae, respectively; $n = 3, 3$ and 8, respectively, for sea scallops

nificantly between early and late postlarval stages of bay scallops (Fig. 3).

While different patterns in esterase and protease activity were exhibited over development by these 2 species, the ontogenetic changes in the esterase: protease ratio were strikingly similar (Fig. 4). This ratio exhibited a ~5- to 7-fold decrease between larvae and immediate postlarvae in both species, with values of 6.5 to 8.3 decreasing to 1.1 to 1.2. This was followed by a period of relative increase from sizes of ~320 to 525 μm until scallops attained ~1200 μm in SH; this ratio remained relatively constant thereafter.

Specific activities of the 3 carbohydrases varied over development; the most pronounced changes occurred before scallops attained ~1.0 to 1.2 mm SH

Fig. 4. *Argopecten irradians*, *Placopecten magellanicus*. Specific activities (see Fig. 3) of esterase and protease, and the ratio of esterase:protease, over development for bay scallops *A. irradians* and sea scallops *P. magellanicus*. Values represent means ($\pm$ data range), $n = 2$ tanks for each sampling size

(Fig. 5). In bay scallops, α -amylase steadily decreased across the 3 developmental stages (48.9 to 35.3 U g^{-1} protein), while there was a 3-fold increase from 7.1 to 22.8 U g^{-1} protein between larval and early postlarval stages in sea scallops, which remained constant into the late postlarval stage (Fig. 3). A steady and significant decrease in cellulase activities of bay scallops was observed (92.5 to 40.1 U g^{-1} protein), while this activity remained constant among these 3 developmental stages in sea scallops. Specific activities of laminarinase increased between larval and postlarval stages in both scallop species ranging from 73.9 to 184.4 U g^{-1} protein in bay scallops and from 109.3 to 308.3 U g^{-1} protein in sea scallops. Overall, the weight-specific ontogenetic patterns observed for the 3 carbohydrates (data not shown) were comparable to those expressed per gram protein (Figs. 3 & 5). Regardless of the units used, amylase

Fig. 5. *Argopecten irradians*, *Placopecten magellanicus*. Specific activities (see Fig. 3) of the carbohydrases α -amylase, cellulase and laminarinase over development in bay scallops *A. irradians* and sea scallops *P. magellanicus*. Values represent means ($\pm$ data range), $n = 2$ tanks for each sampling size

and cellulase activities as a function of SH consistently showed higher activities in bay scallops than in sea scallops, while the reverse was observed for laminarinase.

Comparison of specific activity between species

A *t*-test failed to detect a difference in specific activities of esterase between species when these were averaged across all developmental sizes tested ($p > 0.05$). However, there was a significant difference in protease activity between species with higher values identified in bay scallops ($p < 0.05$). All 3 carbohydrases showed highly significant differences in activity between the 2 scallop species ($p < 0.001$; Fig. 6A). It is noteworthy that bay scallops exhibited specific activities of α -amylase and cellulase almost twice

Fig. 6. *Argopecten irradians*, *Placopecten magellanicus*. (A) Mean specific activities (see Fig. 3) of 3 carbohydrases (α -amylase, cellulase and laminarinase) from whole body homogenates of bay scallops *A. irradians* and sea scallops *P. magellanicus*. (B) Ratios of laminarinase:amylase activities from whole body homogenates of bay and sea scallops, expressed per dry (lyophilized) weight or per gram soluble protein. Values represent means of all developmental sizes ($\pm$ SE); $n = 17$ for bay scallops and 16 for sea scallops. Differences between species determined by t -test; *** $p < 0.001$

as high (38.8 and 56.8 U g⁻¹ protein) as those determined for sea scallops (18.5 and 27.1 U g⁻¹ protein), while the mean laminarinase activity of sea scallops was approximately twice as high as that found in bay scallops (Fig. 6A; 250.6 vs. 142.6 U g⁻¹ protein). Laminarinase:amylase ratios were significantly (~4-fold) higher in sea scallops than in bay scallops, regardless of whether activity was expressed relative to grams protein or grams freeze-dried tissue mass (Fig. 6B). Similarly, laminarinase:cellulase ratios were ~3-fold higher than those measured for bay scallops irrespective of the units used (activity per gram protein or per gram freeze-dried tissue mass).

DISCUSSION

Both scallop species grew exponentially during the experiment, indicating a normal pattern of growth and development. Daily shell growth rates met or exceeded growth rates previously recorded for *Argopecten irradians* (Lu & Blake 1996, Milke et al. 2006) and *Placopecten magellanicus* (Parsons et al. 1993, Milke et al. 2004) of similar size. Variable survival, however, was observed between cohorts of bay scallops. Therefore, examination of digestive enzyme

activity among additional cohorts could be of value in establishing the range of possible variation in this parameter over ontogeny.

Different ontogenetic patterns of enzyme activity were observed both among different enzymes tested and between scallop species. It is possible that changes in the specific enzyme activities over development could have been affected by changes in soluble protein content in the samples; however, patterns of enzymatic activity were very similar when plotted on a per protein or per dry weight basis. This was also reflected in the comparable laminarinase:amylase or cellulase ratios obtained using these 2 units. Although we cannot rule out the possibility that lyophilization of samples prior to homogenization may have reduced enzymatic activities, this would only affect our observed ontogenetic patterns in the 2 scallop species if the sample treatment showed species-, enzyme-, or stage-specific effects.

The variable ontogenetic pattern of enzyme activity observed in the present study contrasts with the steady increase in specific activities of α -amylase over ontogeny in postlarval prawn, *Fenneropenaeus indicus* (Ribeiro & Jones 2000) and early stages (gold embryo to intermolt Juvenile Stage V) of the lobster *Homarus americanus* (Biesiot & McDowell Capuzzo 1990), which might be expected if digestive capacity is correlated with age and associated morphological and functional changes of feeding organs. The assays measured total activity of each enzyme and may include activities in tissues not related to digestion. However, proteolytic activities in non-digestive tissues are most probably lysosomal-derived cathepsin, which are cysteine proteinases and are not likely to be active under the oxidative assay conditions used (Barrett et al. 1998). There is limited information on the lysosomal contribution to the carbohydrase activities examined in our study.

Sampling times were chosen to encompass major developmental changes in the functional morphology of the feeding organs, especially the gills, in the 2 scallop species (Barré 2001, Veniot et al. 2003), which may also be associated with changes in digestive capacity. Prior to 500 μ m SH, postlarval bay scallops have a rudimentary gill, with reflection of the

inner demibranch occurring at ~600 μm SH, and a fully formed gill at 3.3 mm (Barré 2001). Although more protracted, gill development in sea scallops followed a similar progression, with gill reflection occurring around 1 mm SH (Veniot et al. 2003). Reflection of the outer gill demibranch in sea and bay scallop postlarvae coincided with a marked increase in the rate of proliferations of gill filaments, suggesting that this may represent a critical transition during development (Barré 2001, Veniot et al. 2003). Additionally, Kingzett (1993) found that there was a sharp increase in particle clearance rate of the scallop *Patinopecten yessoensis* at ~600 μm , the size of gill reflection in this species. Changes in enzymatic activity between early and late postlarval stages in the present study may thus correspond with a change in feeding function associated with increased feeding efficiency, in turn linked to major transitions in development of the gill and associated feeding organs. We speculate that gut morphology, as well as digestive enzyme activity, may develop in parallel with the above changes, although this remains to be determined.

Bay and sea scallops exhibited remarkably similar and distinct ontogenetic patterns in the ratio of esterase:protease activity, indicating a shift in the need for lipase activity in pediveliger larvae to protease activity in postlarvae. Lipid reserves, especially triacylglycerols (TAG), are considered essential for the metamorphic success of bivalve larvae (Gallager & Mann 1986, Gallager et al. 1986), including sea scallops (Pernet et al. 2006). Therefore, high esterase activities prior to and during metamorphosis may facilitate the catabolism of this energy substrate. The high protease activities evidenced by postlarval scallops relative to larvae in the present study may reflect the high protein demand required to maintain rapid post-metamorphic growth. Protein sparing to meet growth demands has been well documented in mussels, *Mytilus* spp. Thus, conservation of dietary protein for growth is consistent with high oxygen consumption rate/nitrogen excretion rate (O/N) ratios of juvenile *M. trossulus* fed a low protein diet (Kreeger & Langdon 1993), and with the high (75%) maximum efficiency of protein recycling (defined as the percent of protein breakdown products recycled to protein synthesis) measured in juvenile *M. edulis* (Hawkins et al. 1989). High relative protease activity during post-metamorphic stages in the present study may thus reflect high protein recycling. It cannot be ruled out, however, that it may be associated with the use of protein as an energy substrate, since, in contrast to mussels and oysters that preferentially use glycogen as their energy source (e.g. Gabbott & Bayne 1973),

pectinids rely to a greater degree on protein catabolism to meet energy demands (Epp et al. 1988).

Mean cellulase activity was higher in bay scallops than in sea scallops, which may be required to utilize detrital material frequently encountered in the former's estuarine habitat, as has been described for the ribbed mussel *Geukensia demissa* (Lucas & Newell 1984). While it is possible that the higher activities of α -amylase and cellulase observed in bay scallops are associated with the higher temperatures to which these animals are adapted and were reared, this is unlikely as the laminarinase activity was approximately twice as high in sea scallops, and a temperature effect would be expected across all enzymes tested. Laminarinase activity has been widely reported across scallop species such as *Argopecten irradians* (Brock et al. 1986), *A. purpuratus* (Fernández-Reiriz et al. 2004), *Placopecten magellanicus*, (Wojtowicz 1972) and *Pecten maximus* (Stark & Walker 1983). In the present study an increase in specific activity of laminarinase was observed over development in both species, and a similar increase was reported between 4 mm and 40 to 50 mm for *P. maximus* (Økland et al. 2001, Reitan 2011), and during development of post-settlement abalone *Haliotis discus hannai* offered a diatom-based diet (Takami et al. 1998). While this increase in laminarinase activity could reflect a developmental transition, it could also occur in response to chrysolaminarin, a polysaccharide found in diatoms (Beattie et al. 1961) which can be hydrolyzed by laminarinase (Hassett 1994).

It is unclear to what degree enzymatic activity is induced by the presence of substrate. Enzymatic activity against cellulose and alginate during postlarval development of the abalone *Haliotis discus hannai* did not require the presence of the corresponding substrate (Takami et al. 1998). Neither acclimation time nor food type had a significant effect on specific carbohydrase activity in adult cockles *Cerastoderma edule* (Navarro et al. 2009), and no difference in laminarinase activity was observed in juvenile *Pecten maximus* under starvation or offered different diets (Reitan 2011). However, other work conducted on *C. edule* has shown a positive correlation between increased organic content of food and the specific activity of cellulase (Ibarrola et al. 1996, 1998) and specific activities of laminarinase decreased during starvation in juvenile venerid clams *Ruditapes decussatus* and *Venerupis pullastra* (Albentosa & Moyano 2008), implying a relationship between food availability and enzymatic activity. While it cannot be ruled out that seasonal variation can influence enzymatic activity, as shown for adult *Mytilus trossulus*

(Kreeger 1993), it is unlikely to have resulted in the ontogenetic patterns observed in the current study.

The present study shows that digestive enzyme activity is variable both over early development and between scallop species. These changes might be related to energetic requirements for metamorphosis as well as postlarval growth and development. Further work is necessary to determine if substrate availability induces enzymatic activity in bay and sea scallops, which may, in fact, be species, stage and/or enzyme specific.

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