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SEASONALITY IN DIGESTIVE-GLAND SIZE AND METABOLISM IN RELATION TO REPRODUCTION IN *HALIOTIS KAMTSCHATKANA*

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ABSTRACT A novel method of isolating digestive gland cells in abalone was used to provide information on the metabolic activity of this gland in *Haliotis kamtschatkana.* Activity, expressed as percent change in Vo_2 of isolated cells before and after the addition of glucose and amino acid substrates, was studied in relation to sex and to seasonal changes in gonad and digestive gland indices. At 3- to 4-mo intervals between May 1995 and July 1996, five collections of 10 adult abalone (equal sexes) were made from the West Coast of Vancouver Island, British Columbia. Each animal's live mass (without shell) was recorded, and its gonad was aspirated from the digestive gland into a known volume (and mass) of seawater. The digestive gland was sliced free of its attachment, weighed, and related to shell-less body mass as percent digestive gland index (DGI). The gonad live mass was determined from the mass of aspirated mix of gonad and seawater, and related to shell-less body mass to give a percent gonad index (GI). Digestive gland cells were prepared and maintained in a special buffer, and their Vo_2's measured in microrespirometers. There was no sex effect on seasonal DGI, but significant seasonal differences in DGIs correlated perfectly with metabolic activity of digestive-gland cells with glucose substrate. Thus, when digestive glands were largest relative to body size, metabolic activity of their cells was greatest. GIs were significantly higher for males than females. There was a significant seasonal effect on GIs, with values being high in springtime before spawning (April to May, 10–11%) and low in winter (December, 6%), but with statistical overlap between these and summer values (July to August, 7–8%). Metabolic response of the digestive gland cells was highest with glucose substrate (75% increase over presubstrate resting levels as compared with 4% for amino acid substrate), reflecting the carbohydrate-based metabolism of abalone.

KEY WORDS: *Haliotis kamtschatkana,* abalone, digestive gland, isolated cell suspension, metabolism, reproductive cycle

INTRODUCTION

Attempts to study the role of the digestive gland in the nutrition and growth of marine molluscs *in vivo* is hampered by slow growth rates of the test animals, by difficulty in gaining access to the organ, and by multiplicity of function in digestion and energy metabolism of the gland. Added to this in abalone and other gastropods is the intimate juxtaposition of digestive gland and gonad, which tends to confound study of either organ separately. We have side-stepped some of these problems by examining digestive gland function of abalones *in vitro,* using an isolated-cell suspension similar to that used with mammals (Buck et al., 1992) and decapod crustaceans (Lallier and Walsh 1992, Toullec et al. 1992). In this approach, which we believe to be unique for molluscs, the digestive gland is freed from its attachment to the gonad and the rest of the body, and the cells separated and maintained in suspension in a buffer solution formulated especially for this purpose. The cell suspension can then be used as a bioassay system to monitor the metabolic activity of the digestive gland in relation to gametogenetic and other seasonal events and to study the effects of different substrates or other conditions on energy metabolism of the gland.

Reproductive cycles in temperate-latitude abalone generally follow the pattern: gametogenesis during winter and early spring, maximally ripe gonads in late spring and early summer, and spawning during summer and early autumn (Boolootian et al. 1962, Webber and Giese 1969, Poore 1973, Shepherd and Laws 1974, Hayashi 1980, Shepherd and Hearn 1983). However, considerable variation in this cycle may exist within and between species and also geographically and temporally (Boolootian et al. 1962, Poore 1973, Shepherd and Laws 1974, Giorgi and DeMartini 1977, Sloan and Breen 1988). Although *Haliotis kamtschatkana* generally has the highest gonad indices (GIs) in late spring to early summer, with spawnings in mid- to late summer (Breen and Adkins 1980), there is evidence in some populations of "dribble-type" spawning throughout the year (Emmett and Johnstone 1985).

The large foot muscle acts as a glycogen store for use during gametogenesis (Webber and Giese 1969, Webber 1970). Hayashi (1983) showed in *Haliotis tuberculata* that the glycogen content of the foot tissue decreases from 40% to 0 as gonad maturation proceeds, and Webber and Giese (1969) and Webber (1970) showed in *Haliotis cracherodii* a drop in the total dry mass of polysaccharide (=glycogen) in the foot from 23% to 6%, the lowest value coinciding with late-summer spawning. In *H. cracherodii,* as polysaccharides are drawn from foot stores, so their concentration increases significantly in the gonad, somewhat more in males than females (males: 5–9% dry mass increase; females: 4.5–7% dry mass increase; Webber 1970). Because the digestive gland is the chief organ involved in these energy transformations in abalone, its greatest relative size and metabolic activity would be expected concomitantly with active gametogenetic processes.

This study examines the seasonal effects of glucose and amino acid substrates on metabolism of the digestive gland cells and correlates this metabolic activity with the relative size of the gonad and digestive gland. The specific hypotheses to be tested are: (1) that glucose substrate will be metabolically used over amino acid substrate, as suggested by the carbohydrate-based nutrition of abalone, and (2) that metabolic activity, as measured by oxygen consumption of isolated cells, will correlate seasonally with the reproductive cycle. Thus, we predict that the digestive gland will be largest and most metabolically active when gametogenetic activity is greatest.

MATERIALS AND METHODS

At 3- to 4-mo intervals between May 1995 and July 1996, five collections of 10 adult *H. kamtschatkana* of equal sex ratio were made from the West Coast of Vancouver Island, British-Columbia, near the Bamfield Marine Station. During the few days between collection and their use in experiments, the animals were kept in a recirculating seawater system (12°C, 32‰) under ambient light conditions and fed on kelp.

Estimates of gonad and digestive gland masses were made as follows. An abalone was kept on ice for 1 h; then, its shell was removed and its live tissue mass was measured. The membrane enclosing the gonad was slit in several places with fine scissors. The exposed gonadal tissue was aspirated from the surface of the digestive gland with a suction tube connected to a vacuum pump and collected into a known volume (and mass) of seawater. Virtually all of the gonad could be collected in this way, leaving the digestive gland intact. We believe this method to give a much more accurate measure of GI than the area-slice method commonly used with abalone (Boolootian et al. 1962, Poore 1973, Hayashi 1980, and others). The combined mass of the aspirated gonad and seawater, after subtracting the mass of the latter, yielded the fresh mass of gonad. The digestive gland was cut from the animal, weighed, and immersed immediately in chilled buffer formulated specially for the maintenance of abalone digestive gland cells (Taylor and Carefoot unpubl.). The digestive gland and gonad live masses, expressed as percentages of original total body mass without shell, respectively, gave digestive gland index (DGI) and GI for each animal.

Details of the buffer formulation and preparation of the isolated cell suspensions can be found in Taylor and Carefoot (unpubl.). However, briefly, the digestive gland was minced apart with razor blades to free the individual cells and the resulting cell-buffer slurry was filtered through successively finer nylon meshes (to 73 μm pore size), then washed, and centrifuged three times. After the final spin, the cells were resuspended in fresh buffer and rested overnight at 3°C. The overnight buffer differed from the preparation buffer in its lack of glucose and bovine serum albumin. The next morning, a resuspension of the cells in the second buffer provided the stock material for use in the bioassay metabolism experiments. Trypan blue staining tests of these suspensions (Buck et al. 1992) showed +99% viability of the digestive gland cells.

Tests of metabolic response of the cell suspension were done in a 2-mL volume Gilson oxycell (Middleton, WI) equipped with a Clark type O_2 electrode (Yellow Springs, OH). A known mass of cells in fresh buffer was allowed to equilibrate at 15°C for 1 h and was then placed in the oxycell. Baseline Vo_2 of the cells at 15°C was measured over an initial 15-min period, then a nutrient substrate was added, and Vo_2 was measured for an additional 5 min. The difference in before and after rates, expressed as μg min^{-1}g live $cells^{-1}$, gave the measure of metabolic response to a certain substrate. The two substrates tested were glucose and a mixture of amino acids, administered in 50-μL volumes (glucose, 100 mM; amino acids, saturated solution of casein amino acids; ICN, Inc.). Each test of a substrate was done in triplicate.

RESULTS

Seasonal changes in DGIs and GIs are shown in Figure 1. Because no sex effect on digestive gland size was evident ($F[1,49] = 0.53$, $p = 0.47$, analysis of variance [ANOVA]), the values presented for this organ represent males and females combined. A strong seasonal effect on DGI ($F[4,49] = 9.59$, $p < 0.001$, ANOVA) resolved into a generally low value in winter (December 1995; 7.4%) and higher values in summer (August 1995 and July 1996, 9.4 and 12.2%, respectively), although some statistical overlap was present between these and spring values ($p < 0.05$, Newman-Keuls Multiple Comparison Tests). GI also showed a strong seasonal effect ($F[4,35] = 4.41$, $p = 0.005$, ANOVA), with lowest values being found in spawned-out animals in late summer through winter (July, August, December, 6.2–8.6%) and highest values in spring before spawning (April to May, 10.0–11.3%; $p < 0.005$, Neuman-Keuls Tests). Figure 1a also shows a strong sex effect on GI ($F[1,49] = 9.72$, $p = 0.004$, ANOVA). Males with mean GIs of 10% were significantly higher than females with mean GIs of 7% ($p < 0.05$, Neuman-Keuls Tests). There was no significant correlation, either positive or negative, of DGI and GI values over time ($r_s < 0.3$, $p > 0.20$, Spearman Rank Correlation Analysis).

Figure 2 shows the seasonal effect of different substrates on the metabolism of isolated cell suspensions of digestive glands of *H. kamtschatkana*. Sex had a strong effect on the metabolic response of the cells ($F[1,99] = 23.9$, $p < 0.0001$), with females exhibiting the greatest response (overall increases of 78 and 54 μg of O_2 min^{-1} g $cells^{-1}$ over resting rates, respectively, for females and males; $p < 0.05$, Neuman-Keuls Tests). The cells responded strongly to glucose substrate (75 μg of O_2 min^{-1} g $cells^{-1}$ overall increase over resting rate) and only weakly to the amino acid mix

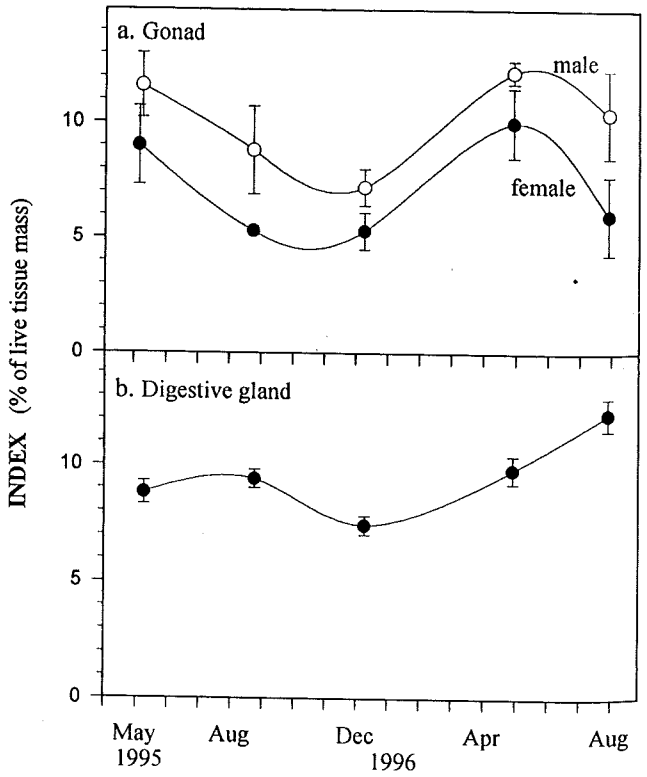

Figure 1. Change in (a) GIs and (b) DGIs over the period May 1995 to August 1996 in *H. kamtschatkana*. Values represent means ± SE of 5 individuals for gonad and 10 individuals for digestive gland. The error bars for August 1995 female GI are hidden within the dimension of the point.

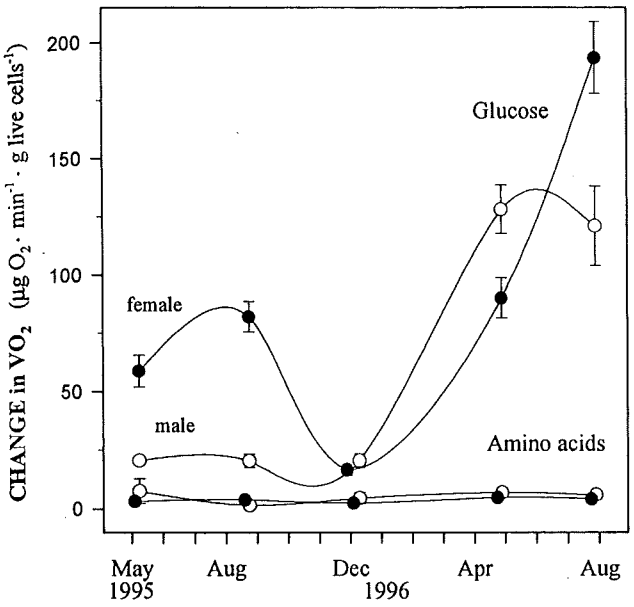

Figure 2. Change in VO_2 of isolated cell suspensions of *H. kamtschatkana* digestive glands from baseline levels when given either glucose or an amino acid mix as substrate. Closed circle, female; open circle, male. Values represent means ± SE of five individuals. Error bars for several of the points are too small to extend beyond the dimension of the points.

(4 μg of O_2 min^{-1} g $cells^{-1}$ increase), with the difference being highly significant ($F[1,99] = 48.2$, $p < 0.0001$, ANOVA). The cells were seasonally least responsive in winter and most responsive in spring and summer ($F[4,99] = 87.0$, $p < 0.0001$, ANOVA), especially spring and summer 1996. These last values formed a statistically segregated grouping from both the winter 1995 values and the spring/summer 1995 values (Fig. 2, $p < 0.05$, Neuman-Keuls Tests).

A perfect correlation was demonstrated between size of digestive gland (DGI) and metabolic response of the cells to glucose ($r_s = 1.0$, Spearman Rank Correlation Analysis). Thus, when digestive glands were smallest (in winter 1995), metabolic response was least, and when the digestive glands were largest (in summer 1996), metabolic response was greatest.

DISCUSSION

GIs in reproductively mature abalone range generally from 12 to 20% of the soft body mass (Webber and Giese 1969, Webber 1970, this study). Although some species spawn more or less completely, leading to indices of essentially zero (e.g., *Haliotis cyclobates* and *Haliotis laevigata* in Australia: Shepherd and Laws 1974), others show less dramatic or no significant seasonal changes (e.g., *Haliotis rufescens* in California: Young and DeMartini 1970, *Haliotis ruber* in Australia: Shepherd and Laws 1974), and GI for these species is of little use in monitoring gametogenetic or spawning activity (Young and DeMartini 1970). In this study on *H. kamtschatkana,* GIs for both males and females showed summer lows, corresponding with spawning, but values remained above about 6%. Because seasonal highs were only 9 and 11% for females and males, respectively, spawning was neither complete nor of high magnitude during 1995 to 1996 for this species.

The greater metabolic responsiveness of female digestive gland cells as compared with those of males in this study cannot be explained simply on the basis of greater gametogenetic growth in females, because gonads in each sex increased by about the same amount during spring/summer 1996. However, Webber (1970) has shown that immediately before spawning the female gonad in *H. cracherodii* consists of about 35% dry mass lipids, as compared with only about 10% for males. In *H. kamtschatkana,* this is reflected in a higher caloric content of female gonads as compared with males (Donovan and Carefoot unpubl.). Lipids represent a much more energetically costly substance to produce than either protein or carbohydrate, and this may at least partly explain the different metabolic activity of the digestive glands in the two sexes.

Our results did not show a clear relationship between digestive gland size and gonad size in *H. kamtschatkana.* Indeed, the few past studies in which DGIs and GIs were measured simultaneously offer somewhat varying views of their interrelationship. Thus, Boolootian et al. (1962) found a strong inverse correlation of digestive gland size with gonad size in *H. cracherodii* and a less evident but nonetheless reciprocal relationship in *H. rufescens.* Webber (1970) also showed DGIs and GIs to be inversely correlated in *H. cracherodii,* but to a much lesser extent than shown by the data of Boolootian et al., and we showed no relationship in *H. kamtschatkana.* Boolootian et al. (1962) interpreted their data for *H. cracherodii* to mean that the digestive gland stockpiles nutrients for gametogenesis, but this was later shown by Webber (1970) not to be the case for this same species, nor would it make sense based on our data for *H. kamtschatkana.* There are two possible explanations for these discrepancies. The first, proposed initially by Boolootian et al. (1962), suggests that the extent and timing of interrelationships of digestive gland and gonad are governed by the seasonal preciseness of gametogenesis and spawning in a species. Thus, *H. cracherodii,* with a distinct seasonal spawning cycle, would have a more marked interrelated cycling of digestive gland and gonad than *H. rufescens* or *H. kamtschatkana,* which exhibits much less distinct spawning cycles (this study, see also Young and DeMartini 1970). The second, less an explanation than a comment, reminds us that digestive gland size and activity will be related not only to gametogenesis, but also to other processes of nutrition and growth. In temperate-latitude species, especially, summer will be a time of optimal feeding and growth, with maximal elaboration of enzymes and allocation of energy for digestion, processing of nutrients, and functioning of transport systems and metabolic pathways involved in all growth, including gametogenesis. Garnering of glycogen and other nutrients required for later gametogenesis may actually be competitive with needs for somatic growth, including that of the shell. Thus, it may be necessary to factor in all growth and activity needs to get a clear picture of seasonal DGI and GI interrelationships in abalone.

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LITERATURE CITED

Boolootian, R. A., A. Farmanfarmaian & A. C. Giese. 1962. On the reproduction cycle and breeding habits of two western species of *Haliotis*. *Biol. Bull.* 122:183–193.

Breen, P. A. & B. E. Adkins. 1980. Spawning in a British Columbia population of northern abalone, *Haliotis kamtschatkana*. *Veliger.* 23:177–179.

Donovan, D. & T. H. Carefoot. 1997. Effect of activity on energy allocation in the northern abalone, *Haliotis kamtschatkana*. *J. Shellf. Res.* (in rev.).

Giorgi, A. E. & J. D. DeMartini. 1977. A study of the reproductive biology of the red abalone, *Haliotis rufescens* Swainson, near Mendocino, California. Calif. *Fish Game.* 63:80–94.

Hayashi, I. 1980. The reproductive biology of the ormer. *Haliotis tuberculata*. *J. Mar. Biol. Assoc. U.K.* 60:415–430.

Hayashi, I. 1983. Seasonal changes in condition factors and in the C:N ratio of the foot of the ormer, *Haliotis tuberculata*. *J. Mar. Biol. Assoc. U.K.* 63:85–95.

Lallier, F. H. & P. J. Walsh. 1992. Metabolism of isolated hepatopancreas cells from the blue crab (*Callinectes sapidus*) under simulated postexercise and hypoxic conditions. *Physiol. Zool.* 65:712–723.

Poore, G. C. B. 1973. Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda) 4. Reproduction. *N. Z. J. Mar. Freshwater Res.* 7:67–84.

Shepherd, S. A. & W. S. Hearn. 1983. Studies on southern Australia abalone (genus *Haliotis*). IV Growth of *H. laevigata* and *H. ruber*. *Aust. J. Mar. Freshwater Res.* 34:461–475.

Shepherd, S. A. & H. M. Laws. 1974. Studies in southern Australian abalone (genus *Haliotis*) II. Reproduction of five species. *Aust. J. Mar. Freshwater Res.* 25:49–62.

Sloan, N. A. & P. A. Breen. 1988. Northern Abalone, *Haliotis kamtschatkana*, in British Columbia: Fisheries and Synopsis of Life History Information. Canadian Special Publication Fish Aquatic Science No. 103. Department of Fisheries and Oceans, Ottawa, 46 pp.

Taylor, B. E. & T. H. Carefoot. 1997. A method of studying digestive-gland metabolism in abalone involving isolated cell-suspensions. (in prep.).

Toullec, J. Y., M. Chikhi & A. Van Wormhoudt. 1992. *In vitro* protein synthesis and alpha amylase activity in F cells from hepatopancreas of *Palaemon serratus* (Crustacea, Decapoda). *Experentia.* 48:272–277.

Webber, H. H. 1970. Changes in metabolic composition during the reproductive cycle of the abalone *Haliotis cracherodii* (Gastropoda: Prosobranchiata). *Physiol. Zool.* 43:213–231.

Webber, H. H. & A. C. Giese. 1969. Reproductive cycle and gametogenesis in the black abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). *Mar. Biol.* 4:152–159.

Young, J. S. & J. D. DeMartini. 1970. The reproductive cycle, gonad histology, and gametogenesis of the red abalone, *Haliotis rufescens* (Swainson). *Calif. Fish Game.* 56:298–309.