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Predator deterrence and 2,4-dibromophenol conservation by the enteropneusts *Saccoglossus bromophenolosus* and *Protoglossus graveolens*

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ABSTRACT: *Saccoglossus bromophenolosus* King et al., 1994 and *Protoglossus graveolens* Giray & King, 1996 contain high concentrations of 2,4-dibromophenol (DBP), the function of which is uncertain. Mature enteropneusts that were collected from the field and maintained *in vitro* without bromide retained DBP, which is inconsistent with active DBP secretion into the burrow environment. DBP was also conserved during field manipulations that decreased food availability *in situ*. Further, DBP did not deter predation in feeding experiments with the anomuran crab *Pagurus longicarpus* and the polychaetes *Glycera dibranchiata*, *Nereis virens* and *Nephtys incisa*. The hermit crabs fed on *S. bromophenolosus* readily, and in preference to shrimp, in the field and in laboratory aquaria. Elevated DBP levels were measured in crabs that had recently consumed *S. bromophenolosus*, and ingested DBP was degraded to 4-bromophenol. Elevated levels of DBP in polychaetes were associated with the disappearance of enteropneusts during *in vitro* feeding experiments. Control incubations with DBP-containing agar plugs indicated that the polychaetes did not accumulate DBP passively. These results suggest that DBP is not an effective anti-predatory agent against hermit crabs or some predatory polychaetes. A definitive role for DBP in enteropneusts remains to be shown.

KEY WORDS: Enteropneusts · Bromophenols · Bromine · Chemical defense · Predation · Polychaetes · Hermit crabs · Starvation

INTRODUCTION

A variety of bromophenols have been extracted from enteropneusts (see King 1986, Woodin et al. 1987, Corgiat et al. 1993, King et al. 1995). Similar compounds have been described for many other marine taxa (see Neidleman & Geigert 1986), most of which are believed to synthesize them via haloperoxidases (e.g. Ahern et al. 1980, de Boer et al. 1986, Manthey & Hager 1989, Chen et al. 1991). The functional roles of these compounds are uncertain, but microbial control (e.g. Sheikh & Djerassi 1975, King 1986, 1988, Goerke et al. 1991) and predation defense (Thomas 1972, Prezant et al. 1981) have been proposed for enteropneusts.

DBP (2,4-dibromophenol) occurs in 2 enteropneusts, *Saccoglossus bromophenolosus* Giray & King, 1996 and *Protoglossus graveolens* King et al., 1994, at average tissue concentrations of 44 and 82 µmol g^{-1} dry wt, respectively. *S. bromophenolosus* and *P. graveolens* co-occur in the intertidal zone of Lowes Cove, Maine, USA (densities of about 100 and 10 ind. m^{-2}, respectively) along with 3 polychaetes, *Glycera dibranchiata*, *Nephtys incisa* and *Nereis virens*, and the anomuran crab *Pagurus longicarpus*. Neither the crab nor polychaetes are known enteropneust predators, although the crab is carnivorous and the polychaetes omnivorous (Commito 1982, Ambrose 1984, Esselink & Zwarts 1989, Redmond & Scott 1989, Volvenko 1994).

Bromophenols have also been described from several polychaetes, some of which contain concentrations comparable to values found in enteropneusts (Higa & Scheuer 1975, Woodin et al. 1987, Goerke & Weber 1990, 1991). However, DBP in *Nereis succinea* (King 1986) and *Glycera dibranchiata* (Woodin et al. 1987) is nearly 1000-fold lower than levels in enteropneusts (King 1986, Higa et al. 1987). Low DBP concentrations in the former may reflect a dietary source consistent with carnivory or omnivory.

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Low bromophenol concentrations have been observed in burrow wall sediments of *Saccoglossus bromophenolosus* and *Protoglossus graveolens* (40 and 600 ng g^{-1} dry wt, respectively; Giray & King 1997). While bromophenols directly affect certain bacteria and other taxa (King 1986, 1988, Jensen et al. 1992, Giray & King 1997), several studies suggest that the effects of bromophenols on bacterial biomass and production are minimal (Steward et al. 1992, 1996). In addition, no specific data document continuous or extensive bromophenol excretion into the burrow.

Data presented here show here that *Saccoglossus bromophenolosus* and *Protoglossus graveolens* conserve DBP rather than excrete it. This is inconsistent with a major role for bromophenols in the burrow environment. In addition, hermit crabs consume *S. bromophenolosus* readily, and several polychaetes accumulate DBP in a manner consistent with predation. While these results do not mitigate against a role in predation defense, it is evident that DBP does not deter some predators, and that predation may play a role in enteropneust population dynamics.

MATERIALS AND METHODS

DBP conservation. *Saccoglossus bromophenolosus* DBP was analyzed by maintaining the enteropneusts *in vitro* in fine sand with 3 treatments: artificial seawater with 0.7 mM bromide (Parsons et al. 1984); artificial seawater with no added bromide; a flow-through system with ambient seawater of 32‰ salinity and estimated bromide content of 0.7 to 0.8 mM. All treatments were maintained at ambient field temperature, aerated and covered to reduce evaporation. The artificial seawater in each treatment was replaced monthly. Plastic containers with 350 cm^3 of fine sand were used as small aquaria. They were submerged under several cm of the appropriate seawater treatment in large trays. Sand for the bromide-free treatments was fired for 4 h at 550°C in a muffle furnace and washed in order to remove any remaining organic matter and bromine. Aquaria were allowed to equilibrate for 1 wk prior to the addition of worms. Ninety *S. bromophenolosus* were collected from Lowes Cove. Three individuals were placed in each of 10 aquaria, with 10 aquaria used per treatment. During the same period *Protoglossus graveolens* (n = 28) were also held in similar aquaria. However, due to their larger size, individuals were placed separately in each aquarium, and only the flow-through seawater treatment was utilized.

At intervals, 5 to 10 individuals were retrieved from each treatment and incubated overnight in seawater representative of the treatment to allow them to void sediment from their guts. All enteropneusts were extracted individually with hexane for DBP determinations (King et al. 1995). Only the proboscis, collar, branchial region and the first 2 cm of the hepatic region were analyzed, as retrieval of complete specimens was nearly impossible. In addition, changes in specimen size were evaluated by measuring collar width using a computerized image analysis system; tissue weight was determined at each interval before DBP extraction.

Population density and DBP conservation. The potential effect of population density on DBP concentration was evaluated *in situ* at a site in the upper region of Lowes Cove with *Saccoglossus bromophenolosus* densities of 80 to 100 m^{-2}. Experimental manipulations of population density were accomplished using cylindrical open-ended 615 cm^2 plastic chambers driven into the sediment to a depth of 28 to 30 cm; 1 to 3 cm of the lip remained above the sediment surface. The chamber depth precluded *S. bromophenolosus* emigration and immigration. Two chambers at ambient density were used as disturbance controls; all *S. bromophenolosus* in these chambers were excavated and subsequently returned to the same sediment. *S. bromophenolosus* collected from adjacent sediment were added to a third chamber at 6-fold the ambient density. *S. bromophenolosus* were also collected from the lower region of Lowes Cove where they occur at densities of about 20 m^{-2}; these enteropneusts were placed in a fourth chamber at a density of 80 to 100 m^2. Available food resources in the lower region of the cove are substantially higher than levels at the upper region, based on sediment nitrogen and chlorophyll content (Mayer et al. 1985, Mayer & Rice 1992).

Saccoglossus bromophenolosus excavated from the chambers and collected from adjacent sediment were sized before placement in chambers; several were also sacrificed for DBP determinations. All *S. bromophenolosus* were excavated from the chambers at the end of the experimental period (1 yr) for analyses of size, weight and DBP content; enteropneusts from adjacent sediment were also collected and assayed for comparison. Size measurements and DBP concentrations were determined as described previously.

Invertebrate predation on enteropneusts. *Nereis virens*, *Glycera dibranchiata* and *Nephtys incisa* were collected from Lowes Cove and also from Days Cove, where *Saccoglossus bromophenolosus* was less abundant. Several individuals of each were extracted with hexane as described earlier (King et al. 1995). Since sub-surface behavior could not be observed directly, predation was inferred from the accumulation of DBP in polychaetes, and the disappearance of enteropneusts maintained with individual polychaetes in aquaria. For this purpose, plastic containers containing 750 cm^3 each of Lowes Cove sediment were incubated in a flowing seawater system. Five *S. bromophenolosus* were placed

in each of 34 containers and equilibrated for 1 wk. *N. virens* (n = 13), *G. dibranchiata* (n = 12) and *N. incisa* (n = 3) from Lowes Cove were then added singly into the containers; the remaining 6 containers were used as controls for *S. bromophenolosus* survival. After 2 wk, all specimens were removed from each container, and the number of enteropneusts determined. Four each of *N. virens* and *G. dibranchiata* were placed in new aquaria with fresh sediment without *S. bromophenolosus* in order to observe post-incubation changes in DBP levels. The remaining polychaetes were extracted with hexane as before for DBP analysis.

In order to determine whether field DBP concentrations in predators changed in the absence of enteropneusts, 5 additional *Glycera dibranchiata* were collected from Lowes Cove, and placed in sediment without *Saccoglossus bromophenolosus*. The polychaetes were sustained on alternate co-occurring food items (e.g. *Nereis virens, Clymenella torquata*) for a period of 8 mo, at the end of which time they were extracted for DBP analysis as above.

In order to determine whether DBP could be taken up by polychaetes indirectly, 2% agarose plugs of about 25 mm length and 4 mm diameter were prepared in 120 µm nylon mesh tubing; each contained approximately 1.9 µmol DBP. Three plugs (total DBP roughly equivalent to that of 5 *Saccoglossus bromophenolosus*) were buried in each of 10 plastic containers holding 750 cm^3 of sediment from Lowes Cove and maintained in a flowing seawater system. *Nereis virens* (n = 5) and *Glycera dibranchiata* (n = 5) were collected from Lowes Cove and placed individually in the containers. After 2 wk, the polychaetes were retrieved and extracted as above. A parallel analysis was based on the use of *S. bromophenolosus* in the mesh tubes.

The hermit crab *Pagurus longicarpus* (n = 22) was collected from Lowes Cove, placed separately in 950 cm^3 plastic containers on a seawater table and equilibrated for 1 h. One specimen of *Saccoglossus bromophenolosus* and a portion of shrimp tissue of similar size were placed into each of 12 containers, while either *S. bromophenolosus* or shrimp tissue was added to the remaining 10 containers. All treatments were observed for 2 h. At the end of this period, the remaining enteropneusts and shrimp tissue were removed. Predation by *P. longicarpus* was observed directly. After 24 h, one freshly collected specimen of *S. bromophenolosus* was presented to each crab. Seven of the crabs which had consumed *S. bromophenolosus* within the last 24 h, and 6 freshly collected crabs were extracted for DBP analysis. Crabs were first frozen at −80°C, extracted from their shells, then crushed by mortar and pestle. Extractions and gas chromatographic analysis were conducted as before.

In order to observe changes in DBP content in the absence of feeding on enteropneusts, 5 additional *P. longicarpus* which had consumed *S. bromophenolosus* were held in containers and sustained on shrimp for an additional week before extraction. DBP contents were determined by gas chromatography and mass spectroscopic analysis as above. All statistical analyses were performed using ANOVA with Tukey's test at $p < 0.05$.

RESULTS

DBP conservation

No significant differences ($p < 0.05$) in total DBP levels were observed for *Saccoglossus bromophenolosus* maintained in seawater, artificial seawater with bromide or artificial seawater without bromide (Fig. 1A). No signifi-

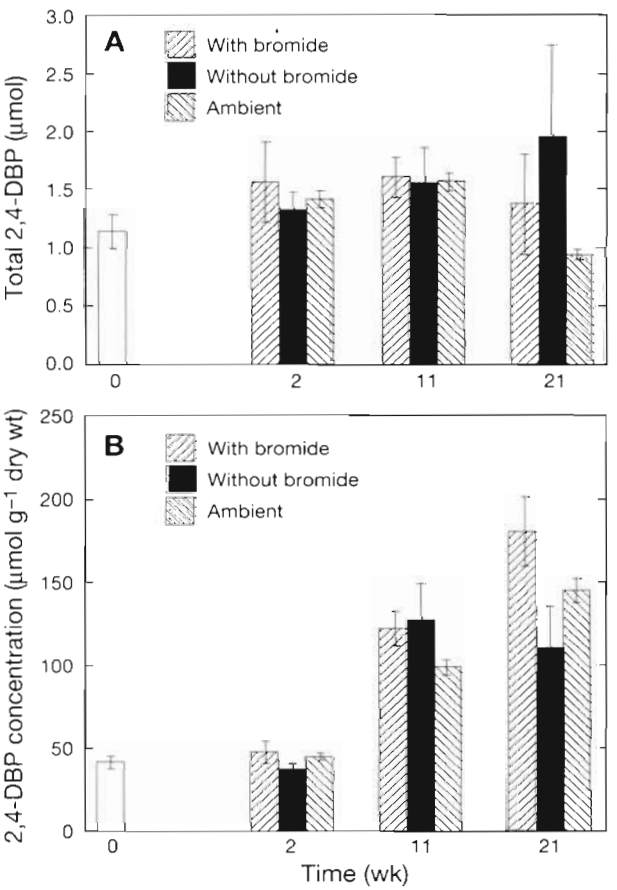

Fig. 1. (A) Total 2,4-dibromophenol (DBP) content in extracts of individual *Saccoglossus bromophenolosus* during incubation in artificial seawater with or without bromide and in a flow-through system of ambient seawater. (B) 2,4-DBP concentration in extracts of individual *S. bromophenolosus* during incubation in artificial seawater with or without bromide and in a flow-through system of ambient seawater; open bars are initial values; data are means ±1 standard error

cant differences ($p < 0.05$) were observed in total DBP levels for similar incubations with *Protoglossus graveolens* (Fig. 2A). However, DBP tissue concentrations increased significantly ($p < 0.05$) in both taxa (Figs. 1B & 2B). In *S. bromophenolosus* maintained with or without bromide, DBP concentrations increased up to 4.4- and 3.1-fold, respectively (Fig. 1B). For *S. bromophenolosus* and *P. graveolens* maintained in ambient seawater, DBP concentration increased 3- to 3.5-fold after the 21 wk incubation period (Figs. 1B & 2B). DBP concentrations increased markedly in the proboscis and trunk regions of *S. bromophenolosus*, but not in the collar (Table 1). Significant increases ($p < 0.05$) in DBP concentration were observed in the proboscis and trunk as well as the collar of *P. graveolens* (Table 1).

The increase in DBP concentration was accompanied by a progressive diminution in size for the experimental animals as indicated by a dramatic decrease in collar dimensions and mass. Collar widths of both *Saccoglossus bromophenolosus* and *Protoglossus graveolens* decreased rapidly and significantly ($p < 0.05$) during the first 2 wk of incubation in all treatments, with no significant change for the remainder of the experiment (Figs. 3 & 4). The decrease in collar size was paralleled by a significant decrease ($p < 0.05$) in mass of *S. bromophenolosus* and *P. graveolens* after 2 and 10 wk, respectively (Figs. 3 & 4).

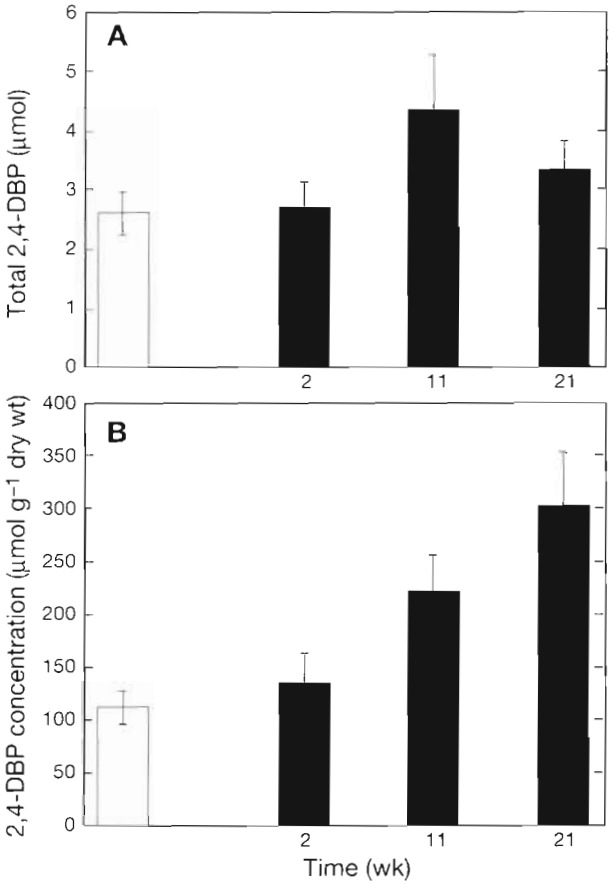

Fig. 2. (A) Total 2,4-dibromophenol (DBP) content in extracts of *Protoglossus graveolens* during incubation in ambient seawater; (B) 2,4-DBP concentration in *P. graveolens* during incubation in ambient seawater; open bars are initial values; data are means ± 1 standard error

Population density and DBP conservation

Recoveries of *Saccoglossus bromophenolosus* from treatments in the upper cove after 1 yr varied between 74% in the high density treatment and 80% in the control chambers. *S. bromophenolosus* collar widths increased relative to starting values for worms in control chambers and adjacent ambient sediment; however, collar widths decreased for worms in the high density treatment ($p < 0.05$; Fig. 5).

Saccoglossus bromophenolosus was recovered almost entirely (98%) from the transplantation treatment. Collar widths of individuals transferred from the lower cove to the upper cove were significantly reduced from values at the beginning of the study ($p < 0.05$). In addition, at the end of the study the collar widths of transplanted individuals were significantly smaller ($p < 0.05$) than those of *S. bromophenolosus* in the ambient sediment in the upper and lower cove, and in control chambers (Fig. 5). Initial and final collar

Table 1. Initial and final concentration of 2,4-dibromophenol (DBP) in proboscis, collar and trunk tissues of *Saccoglossus bromophenolosus* und *Protoglossus graveolens* during the 2,4-DBP excretion study. Final concentrations which differ significantly from initial values are noted by asterisks

Sample	Treatment	2,4-DBP concentration (µmol g^{-1} dry wt) Initial	Final
Saccoglossus bromophenolusus			
Proboscis	Artificial seawater with bromide	110.12 (±9.37)	347.28 (±65.27)*
Collar	Artificial seawater with bromide	10.82 (±3.28)	21.45 (±6.97)
Trunk	Artificial seawater with bromide	26.68 (±6.48)	126.44 (±37.17)*
Proboscis	Artificial seawater w/o bromide	110.12 (±9.37)	288.64 (±43.61)*
Collar	Artificial seawater w/o bromide	10.82 (±3.28)	14.53 (±5.23)
Trunk	Artificial seawater w/o bromide	26.68 (±6.48)	77.24 (±27.18)
Proboscis	Flow-through ambient seawater	110.12 (±9.37)	347.96 (±40.46)*
Collar	Flow-through ambient seawater	10.82 (±3.28)	27.06 (±4.47)
Trunk	Flow-through ambient seawater	26.68 (±6.48)	99.40 (±14.69)*
Protoglossus graveolens			
Proboscis	Flow-through ambient seawater	98.95 (±16.10)	325.98 (±74.43)*
Collar	Flow-through ambient seawater	194.84 (±64.02)	560.84 (±114.12)*
Trunk	Flow-through ambient seawater	110.87 (±14.34)	251.37 (±31.91)*

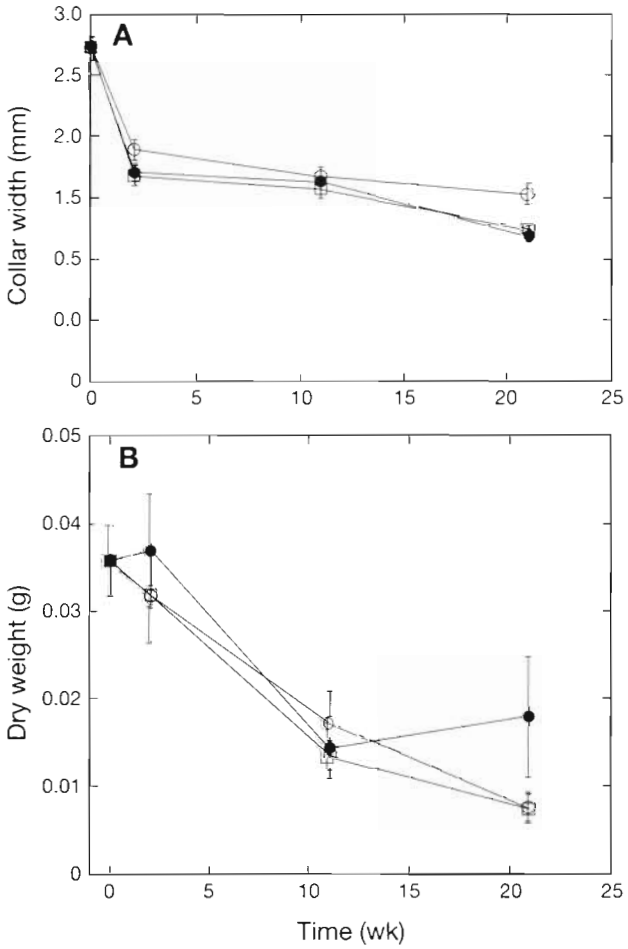

Fig. 3. (A) Change in collar width of *Saccoglossus bromophenolosus* during incubation in artificial seawater with (□) or without bromide (●) and in a flow-through system of ambient seawater (○). (B) Change in dry weight of *S. bromophenolosus* during incubation in artificial seawater with (□) or without bromide (●) and in a flow-through system of ambient seawater (○); data are means ± 1 standard error

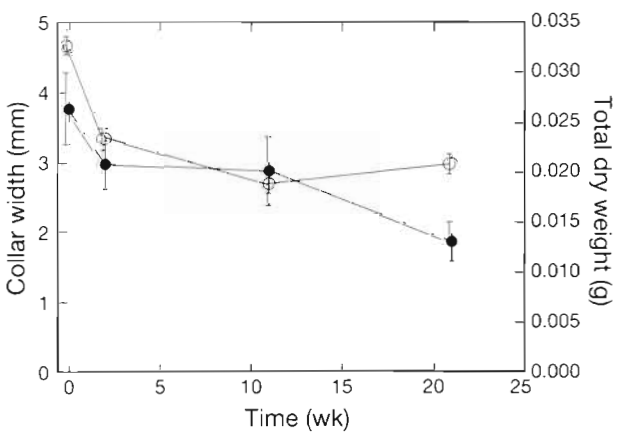

Fig. 4. Collar width (○) and total dry weight (●) of *Protoglossus graveolens* during incubation in ambient seawater; data are means ± 1 standard error

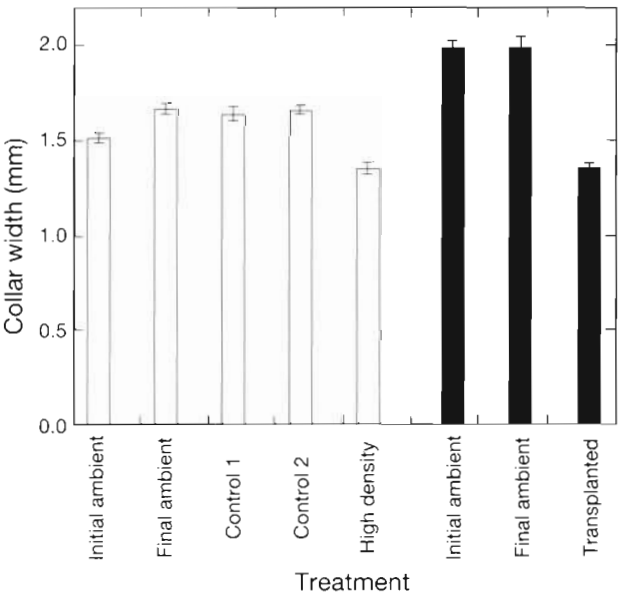

Fig. 5. Change in *Saccoglossus bromophenolosus* collar widths during incubation at high densities and transplantation between sites of low (open bars) and high (filled bars) food levels; data are means ± 1 standard error

widths of *S. bromophenolosus* from ambient sediment in the lower cove were not significantly different, but were significantly larger ($p < 0.05$) than those observed in the upper cove (Fig. 5).

The difference in collar widths between *Saccoglossus bromophenolosus* held in the high density treatment and those collected from ambient sediment was mirrored by their masses: 8.9 ± 1 mg dry wt worm^{-1} for individuals in the high density treatment versus 17.3 ± 2 mg dry wt worm^{-1} for those in ambient sediment. In contrast, DBP concentrations were significantly higher in individuals from the high density treatment relative to levels in worms from ambient sediment (Fig. 6). DBP levels in *S. bromophenolosus* from ambient sediment did not differ significantly ($p < 0.05$) from the beginning to the end of the study (Fig. 6).

Invertebrate predation on enteropneusts

DBP was detected in extracts from 9 of 19 *Nereis virens*, 8 of 11 *Glycera dibranchiata* and in only 1 of 5 *Nephtys incisa* collected from Lowes Cove. Although the highest concentrations of DBP were detected in *G. dibranchiata* and *N. incisa*, comparisons of average DBP concentrations did not show statistically significant ($p < 0.05$) differences among the 3 species (Fig. 7).

Nearly 100% of *Saccoglossus bromophenolosus* placed in control containers were recovered at the end of the predation experiment. However, in containers

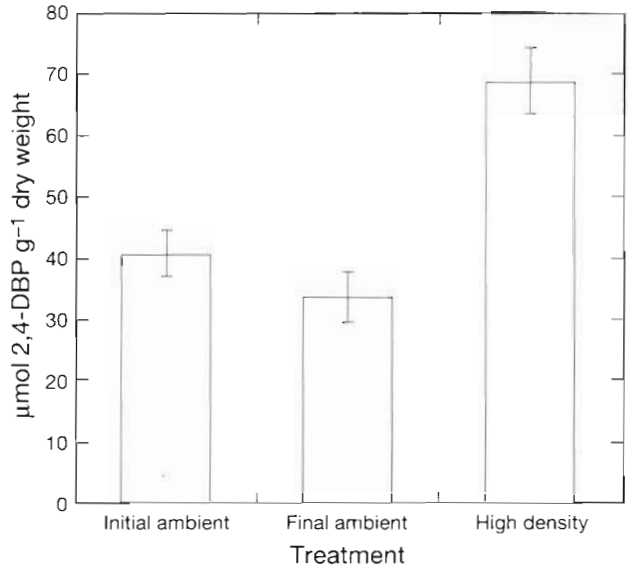

Fig. 6. 2,4-dibromophenol (DBP) concentration in *Saccoglossus bromophenolosus* during incubation in the field at high densities; data are means ± 1 standard error

where *S. bromophenolosus* were incubated with predatory polychaetes, recoveries were 46, 56 and 40% for *Nereis virens*, *Glycera dibranchiata* and *Nephtys incisa*, respectively.

Nereis virens, *Glycera dibranchiata* and *Nephtys incisa* incubated with *Saccoglossus bromophenolosus* for 2 wk had higher DBP concentrations than freshly collected individuals from Lowes Cove (Fig. 7). DBP

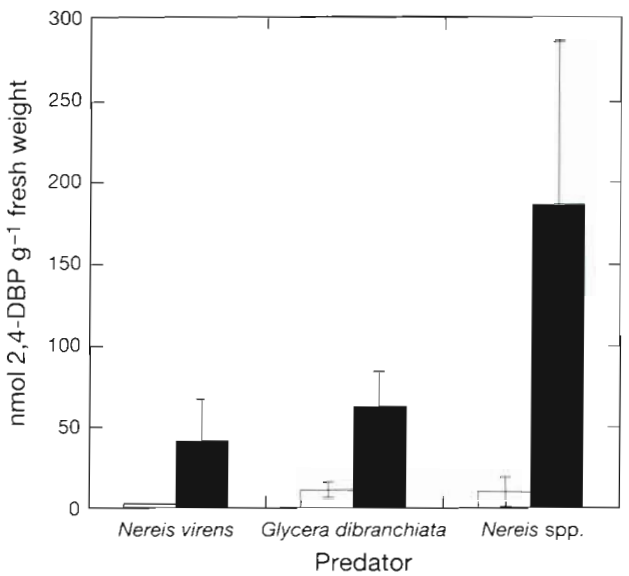

Fig. 7. Naturally occurring (open bars) and post-incubation (filled bars) 2,4-dibromophenol (DBP) concentrations in 3 predatory polychaete species from Lowes Cove; data are means ± 1 standard error

concentrations increased by 9- to 10-, 4- to 5-, and 6-fold for the 3 respective species; the greatest absolute increase occurred in *N. incisa* (Fig. 7). The increases in DBP concentration were statistically significant for all 3 polychaete species ($p < 0.05$). Among the 3 polychaete species, the lowest DBP concentrations occurred in *N. virens*, both during incubations with *S. bromophenolosus* and in freshly collected individuals (Fig. 7). DBP levels did not correlate with the number of *S. bromophenolosus* missing from each aquarium at the end of the experiment. DBP was not detected in *N. virens* maintained in sediment with either DBP-containing agarose plugs or *S. bromophenolosus* in mesh tubes. *G. dibranchiata* maintained in the same manner contained DBP levels (1.7 ± 1.2 nmol g^{-1} fresh wt) that were significantly below ($p < 0.05$) those measured in freshly collected polychaetes.

DBP concentrations dropped to ambient levels in *Glycera dibranchiata* that were used in the feeding experiment after incubation in the absence of *Saccoglossus bromophenolosus* for 1 mo, and were undetectable after 8 mo. DBP was undetectable after 1 mo in *Nereis virens* that were incubated similarly.

The average concentration of DBP in tissues of *Glycera dibranchiata* collected from Days Cove (14.2 nmol g^{-1} fresh wt) was not significantly different from concentrations measured for specimens collected from Lowes Cove ($p < 0.05$). However, DBP was detected in only half of the individuals from Days Cove in contrast to 75% of those from Lowes Cove. None of the *Nereis virens* collected from Days Cove contained detectable levels of DBP.

Field observations indicated that freshly collected *Saccoglossus bromophenolosus* were readily consumed when placed several cm away from hermit crabs *Pagurus longicarpus*. During laboratory feeding studies, hermit crabs consumed *S. bromophenolosus* in preference to shrimp: 8 of 12 *P. longicarpus* consumed *S. bromophenolosus* rather than shrimp; 1 consumed shrimp but not *S. bromophenolosus*; the remaining 3 took neither food item. When presented separately, *S. bromophenolosus* and shrimp were both accepted equally by 10 hermit crabs used as controls. All *S. bromophenolosus* were completely ingested; although feeding began immediately, it generally took 2 h before substantial loss of worm tissue was evident, and an overnight incubation was necessary before *S. bromophenolosus* 1.5 × 20 mm in size were completely consumed. *P. longicarpus* which had previously consumed *S. bromophenolosus* readily accepted additional specimens during repeated feedings.

Pagurus longicarpus did not generally contain detectable levels of DBP. Instead, 4-bromophenol (BP) was detected in both freshly collected crabs and crabs fed *Saccoglossus bromophenolosus* (Table 2). DBP was

Table 2. Concentrations of 2,4-dibromophenol (DPB) and 4-bromophenol (BP) in hermit crabs *Pagurus longicarpus*

Treatment	nmol 2,4-DBP (g^{-1} fresh wt)	nmol 4-BP (g^{-1} fresh wt)
Freshly collected from Lowes Cove	None detected	342.78 (±107.6)
24 h after feeding on enteropneusts	26.51 (±3.23)	190.12 (±39.43)
7 d after feeding on enteropneusts	None detected	468.49 (±49.07)

detected only in *P. longicarpus* that had been observed to consume *S. bromophenolosus* within the previous 24 h (Table 2). However, DBP concentrations were less than those of BP (Table 2). The concentration of BP was not significantly different between hermit crabs collected from the field and those that had consumed *S. bromophenolosus* in the laboratory ($p < 0.05$). However, BP levels were significantly greater in crabs that were extracted 24 h versus 7 d after feeding on *S. bromophenolosus* ($p < 0.05$).

DISCUSSION

DBP is conserved, or even concentrated, in enteropneusts during *in vitro* incubations without bromine for periods greater than 5 mo (Figs. 1, 2 & 3). Conservation is inconsistent with the use of DBP for controlling microbial activity in burrow wall sediments, even though there may be incidental effects of DBP on burrow wall microbes (King 1986, 1988, Giray & King 1997). Regulation of microbial activity would require routine DBP excretion, since DBP is labile during anoxic conditions that characterize macrofaunal burrows for extended periods (Kristensen 1985, Kristensen et al. 1991, authors' unpubl. obs.). Rapid recycling of any bromine released during DBP degradation is unlikely to account for observed DBP conservation since diffusion from the burrow microenvironment would inevitably result in significant bromine losses. Conservation in spite of decreased tissue mass (Figs. 3, 4 & 5) further suggests that DBP is not routinely excreted, and that it does not play any important role as an energy or carbon reserve.

Losses of body mass during the *in vitro* incubations (Figs. 3 & 4) indicate insufficient substrate for maintenance. Similar losses occurred during incubations of *Saccoglosus bromophenolosus* at unusually high densities *in situ* (Fig. 5). These incubations also resulted in increased tissue DBP concentrations. Thus, DBP conservation *in vitro* cannot be attributed to a response to bromine deprivation, a sandy substrate or lack of natural food sources. Collectively, the results of *in vitro* and *in situ* incubations support a role other than regulation of the burrow microbiota for enteropneust DBP.

Alternatively, DBP could be maintained at constant or even elevated levels if a halogenated DBP-precursor not measured by our methods is stored and mobilized over time. Although the non-hexane extractable sulfamate of 2,3,4-tribromopyrrole accounts for most of the bromopyrrole content of *Saccoglossus kowalevskii* (Fielman & Targett 1995), we have found no evidence for sulfate esters of bromophenols in *S. bromophenolosus*. Moreover, if such esters were important, they would have to be present in implausably high concentrations (>10% of dry weight) to support significant, routine DBP excretion over months.

Secondary compounds serve as predation defenses (e.g. McEuen 1984, Pawlik et al. 1984, Young et al. 1986, DeMott & Moxter 1991, Fenical & Pawlik 1991, Pawlik & Fenical 1992) or antibacterial agents (e.g. Al-Ogily & Knight-Jones 1977). Previous observations on enteropneusts have been inconsistent with respect to the role of DBP. Thomas (1972) showed that flounder usually rejected *Saccoglossus otagoensis* when presented separately or with other food items in laboratory studies, and concluded that the enteropneust was protected, but not fully immune to predation. Earlier studies noted that *Ptychodera* sp. was rarely found in the alimentary canals of co-occurring predatory fish (Devanesen & Chacko 1942 referenced in Thomas 1972). 2,3,4-tribromopyrrole produced by *S. kowalevskii* also actively repelled small predatory fish and crabs (Prezant et al. 1981). However, other observations have indicated that bromophenols may not be particularly effective as predation defenses; for example, sea birds *Calidris* sp. and a tropical mollusk prey on *Ptychodera flava* (Azariah et al. 1978, M. Hadfield pers. comm.).

Although DBP conservation by *Saccoglossus bromophenolosus* and *Protoglossus graveolens* is consistent with a role in predation defense, the hermit crab *Pagurus logicarpus* readily consumes *S. bromophenolosus*, even in preference to a non-toxic food source (shrimp). Hermit crabs identify food resources by chemoreception (Brooks 1991); this extends to food aversion learning as well (Wight et al. 1990). It is thus plausible that DBP serves as a chemoattractant rather than a deterrent. Enteropneusts are clearly vulnerable to hermit crab predation because they extend their proboscides onto the sediment surface to feed. During feeding, the proboscis may represent an easy target for a variety of surface predators. The facility with which *S. kowalevskii* regenerates its proboscis (Tweedel 1961) is perhaps a response to such predation, as well as an example of escalation in the complexity of defenses during the co-evolution of predators and prey.

Three polychaetes, *Nereis virens*, *Nephtys incisa* and *Glycera dibranchiata*, also appear to prey on *Saccoglossus bromophenolosus* as indicated by the presence of DBP in specimens collected from Lowes Cove, and by significantly ($p < 0.05$) elevated levels of DBP following incubation with enteropneusts (Fig. 7). Losses of *S. bromophenolosus* in containers with polychaetes were presumed to result from either predation or disturbance by the polychaetes. The complete loss of DBP from *G. dibranchiata* and *N. virens* following incubation in the laboratory without *S. bromophenolosus* is also consistent with at least occasional DBP intake via predation. The possibility of incidental DBP uptake by predatory polychaetes appears minimal since incubation of polychaetes with both DBP-containing agarose plugs and *S. bromophenolosus* enclosed in mesh tubes did not result in elevated DBP in polychaete tissues. Thus, our results show that polychaete and crustacean predators do not avoid enteropneusts, but actively select them in the case of hermit crabs.

Since halogenated organic compounds are toxic to a number of organisms (e.g. Kerger et al. 1988, King 1988, Casillas & Myers 1989, Teeyapant et al. 1993), our results indicate that both hermit crabs and polychaetes must possess a mechanism for detoxifying DBP. In hermit crabs, detoxification appears to involve dehalogenation, since a mono-bromophenol (4-BP) was observed in its tissues (Table 2). Dehalogenation of this sort has not been previously reported for animals. In contrast, 4-BP was not detected in any of the polychaetes, which implies that either DBP is excreted as such, or that it is rapidly and completely dehalogenated. The lack of correspondence between polychaete DBP levels in the feeding experiment and the number of enteropneusts presumably ingested suggests a turnover which is rapid enough that DBP concentrations reflect only the most recent feeding activity. The more rapid loss of DBP from *Nereis virens* may indicate that it possesses a more efficient means of detoxifying (or excreting) DBP than other polychaetes. Consequently, low ambient tissue DBP levels in *N. virens* may not correspond to the extent of its predation on *Saccoglossus bromophenolosus*.

In addition to anti-microbial and anti-predator roles, other functions have been suggested for bromophenols and related compounds. For example, a role in larval recruitment has been proposed, since larvae of *Nereis vexillosa* do not settle readily in sediments containing bromophenols secreted by the polychaete *Thelepus crispus* (Woodin 1991, Woodin et al. 1993). Further, recruitment of *Mytilus viridis* larvae is inhibited by secretions of *Ptychodera flava*, which contains bromochloroindoles (Azariah et al. 1978). Bromophenols may also serve as triggering cues during the synchronous release of gametes by at least some saccoglossids (Burdon-Jones 1951, Hadfield 1975). Fielman & Targett (1995) have observed an increase in the level of 2,3,4-tribromopyrrole (TBPy) in the proboscis and tail of *Saccoglossus kowalevskii* during the months of gametogenesis and spawning activity, suggesting a possible role as an anti-predatory agent during this interval.

In a search for the raison d'etre for enteropneust bromophenols, few possibilities can be excluded. Roles in intermediary metabolism and regulation of the burrow wall microbiota seem least likely. Larval recruitment and signaling within or among populations are plausible, but perhaps represent secondary roles. Predation defense remains the most likely selective force for bromophenol accumulation. However, it is evident that mortality resulting from predation by bromophenol-insensitive predators (e.g hermit crabs) may contribute significantly to the dynamics of enteropneust populations, and becomes a selective force for other enteropneust traits (e.g. rapid tissue regeneration). Comparisons of the relative effectiveness of various bromophenols in predation defense and the relationship between tissue concentrations and feeding deterrence will clarify these roles further. For the present however, a definitive role for DBP in enteropneusts has yet to be shown.

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