SEQUESTERED PARALYTIC SHELLFISH POISONING TOXINS MEDIATE GLAUCOUS-WINGED GULL PREDATION ON BIVALVE PREY

RIKK G. KVITEK[1]

Department of Zoology, University of Washington, Seattle, Washington 98195 USA

ABSTRACT.—Glaucous-winged Gulls (*Larus glaucescens*) avoid paralytic shellfish poisoning by conditioned aversions developed after the regurgitation of contaminated bivalve prey. In feeding experiments with free-ranging Glaucous-winged Gull chicks, toxic (445 μg saxitoxin equivalents per 100 g) butter clams (*Saxidomus giganteus*) were regurgitated in <5 min (n = 58), and nontoxic butter clams were never regurgitated (n = 30). Chicks that had ingested toxic *S. giganteus* then refused to eat either toxic or nontoxic butter clams offered later, but they accepted other bivalve prey. In feeding experiments with wild adult and juvenile *L. glaucescens*, gulls took significantly fewer butter clams at a site where *S. giganteus* has been chronically toxic for >14 yr than they took at a nontoxic but otherwise comparable site. These preferences were species-specific and independent of prey toxicity or whether the live clams were intact or removed from their shells. The gulls showed no difference between sites in their preferences for three other bivalve species (*Clinocardium nuttalli, Protothaca staminea, Tresus capax*), all of which contained either no or very low levels of toxins at both sites. The aversion to intact but not to shucked butter clams was more strongly developed in adults than in juveniles. Most of the gulls that ate butter clams at the toxic site discarded the siphons (which account for the majority of the toxicity in contaminated butter clams) of both toxic and nontoxic butter clams, but they never discarded siphons of other bivalves. Gulls at the nontoxic site never discarded the siphons of bivalve prey. I suggest that avian predators generally are not at risk from paralytic shellfish poisoning toxin via bivalve vectors because they can detect and avoid toxic prey. Because of the apparent inability of gulls to discriminate between toxic and nontoxic individuals of the same prey species, avian predation pressure on some bivalve populations may be greatly reduced. *Received 16 July 1990, accepted 8 November 1990.*

PARALYTIC shellfish poisoning (PSP) results from the consumption of food contaminated with highly lethal neurotoxins produced by toxigenic dinoflagellates (*Protogonyaulax* spp.) (Halstead 1978). Paralytic shellfish poisoning toxins (PSPT) can move through marine food chains and have been implicated in the mass mortalities of planktivorous fish (White 1977, 1980, 1981a, b) and seabirds (McKernan and Scheffer 1942, Coulson et al. 1968, Bicknell and Walsh 1975, Sasner et al. 1975, Armstrong et al. 1978, Hockey and Cooper 1980, Nisbet 1983). The most common vector of the toxins in cases of bird mortalities has been planktivorous fish, yet it is suspension-feeding bivalves that present the greatest paralytic shellfish poisoning risk to human health (Quayle 1969, Prakash et al. 1971, Shumway 1990). Toxic "red tides" (blooms of toxigenic dinoflagellates) recur globally, appear to be increasing in frequency, and result in numerous shellfishery closures and human illnesses and deaths every year (Halstead 1978, Nishitani and Chew 1988, Shumway 1990).

Although many marine and coastal birds prey on bivalves (Oldham 1930, Recher 1966, Barash et al. 1975, Sanger and Jones 1982, Vermeer 1982, Bourne 1984, Nettleship et al. 1984, Richardson and Verbeek 1986, Roberts et al. 1989) known to concentrate PSPT (Quayle 1969, Prakash et al. 1971), avian mortalities have only rarely been associated with PSPT contaminated bivalve prey (Bicknell and Walsh 1975, Sasner et al. 1975, Hockey and Couper 1980). Indeed, it is the rarity of PSP-related avian deaths that is most surprising given the extreme lethality of the toxins and their widespread occurrence. Paralytic shellfish poisoning toxins are among the most potent neurotoxins known and are fatal to most

[1] Present address: Moss Landing Marine Laboratories, P.O. Box 450, Moss Landing, California 95034 USA.

vertebrates at extremely small concentrations (Halstead 1978). The World Health Organization has set the maximum PSPT concentration allowed in shellfish for human consumption at 80 μg STX equivalents/100 g. (Paralytic shellfish poisoning toxins are derivatives of saxitoxin [STX], one of the two most lethal PSPT, and mouse bioassay results are expressed in STX equivalents.) In two studies with birds, pigeons (*Columba* sp.) were found to have a LD_{50} of 90 μg STX equivalents/kg (see Halstead 1978), and the lethal dose for European Starlings (*Sturnus vulgaris*) was <490 μg STX/kg (Kvitek and Beitler 1988, 1989). Because bivalve toxicities are routinely well above these levels in many areas, often measured in the thousands of μg STX/100 g (Quayle 1969, Prakash et al. 1971, Halstead 1978, Shumway 1990), either avian survival or their predation on bivalves must be profoundly influenced at these times, unless the birds are highly resistant to PSPT.

Immunity to PSPT is not the most probable explanation for the rarity of bivalve-related PSPT bird mortalities given that PSPT has been implicated in bird kills via other vectors as well as bivalves in a few cases. A more likely hypothesis is that avian predators may develop aversions to toxic bivalve prey. Tufts (1979) cites anecdotal accounts of resident gulls (*Larus* spp.) and eiders (*Somateria* spp.) avoiding toxic shellfish that kill migratory Black Ducks (*Anas rubripes*), and of a duck that regurgitated force-fed toxic mussels. Acquired aversions to toxic prey have been well documented for some insectivorous birds (e.g. Blue Jays, *Cyanocitta cristata*, that encounter monarch butterflies; Brower and Fink 1985). These species have learned to identify and avoid prey that is toxic or likely to be toxic, and in some cases to circumvent the chemical defenses of their prey by selective rejection of the most toxic tissues. Similar responses have been demonstrated in sea otters (*Enhydra lutris*; Kvitek et al. in press) and fish (*Leptocottus armatus*; Kvitek MS) fed PSPT-contaminated bivalves.

If it occurs, avoidance of bivalve and other intertidal invertebrates by avian predators as a result of sequestered PSPT could have significant ecological and economic implications. Shorebirds have been shown to have profound effects on infaunal prey abundance and distribution (O'Conner and Brown 1977, Evans et al. 1979, Quammen 1984) and to prey heavily on commercially important bivalve species (Bourne 1984, Nettleship et al. 1984, Richardson and Verbeek 1986).

Gulls (*Larus* spp.) commonly prey on bivalves by dropping them from aloft to crack open the shell (Oldham 1930, Barash et al. 1975, Roberts et al. 1989). In the Pacific Northwest, Glaucous-winged Gulls (*Larus glaucescens*) frequently forage on infaunal bivalves (*Protothaca staminea, Tapes japonica, Clinocardium nuttalli,* and *Saxidomus giganteus*) (Barash et al. 1975, Vermeer 1982, pers. obs.). Each of these prey species retains PSPT for varying lengths of time (5 weeks to 2 yr) following exposure to a toxic "red tide" (Quayle 1969, Shumway 1990). Red tides are common along the west coast of North America, with many beaches closed to clamming each year (Quayle 1969, Nishitani and Chew 1988), but there have never been reports of gull mortalities associated with toxic shellfish. In Washington State alone, bivalves at >50 sites contained PSPT concentrations above the public health closure level (80 μg STX equivalents/100 g) during 1989 (Washington State Department of Health unpubl. rep.).

My primary purpose was to resolve the paradox of seabirds that prey on bivalves but do not die as a result of PSPT-contaminated prey. I conducted a series of feeding experiments with free-ranging wild Glaucous-winged Gulls to determine (1) if experienced and naive gulls differ in their response to toxic prey, (2) whether or not naive gulls develop a conditioned aversion following the ingestion of toxic prey, and (3) at what level (individual, species, genus or family) conditioned gulls discriminate between prey, if an aversion is formed. I chose butter clams as the toxic prey because of the availability of highly toxic individuals due to this species' tendency to retain PSPT for >1 yr (Quayle 1969, Shumway 1990). They are also abundant in many intertidal habitats and thus frequently eaten by Glaucous-winged Gulls and other birds (Barash et al. 1975, Vermeer 1982, Vermeer and Bourne 1984, pers. obs.). I selected three sites for the experiments. One was a Glaucous-winged Gull breeding area that does not support infaunal bivalve prey as forage for gulls; the second, an area heavily used by foraging Glaucous-winged Gulls where butter clams but not all bivalves are chronically toxic; and the third, a similar but nontoxic site as a control. In this way I was able to compare the feeding behavior of birds chronically exposed to PSPT-laden butter clams with that of naive chicks at

TABLE 1. Bivalve communities at Scatchet Head (nontoxic site) and Middle Ground (toxic site). Toxicity levels are given for bivalves used in gull feeding experiments; NDT = no detectable toxin (<37 μg STX equivalents/100 g). All samples were analyzed with mouse bioassay, except the cockles, which were tested with high performance liquid chromatography. Means ± SD are given for bivalve density (n = 5 and 6 for Scatchet Head and Middle Ground, respectively).

	Scatchet Head (nontoxic)		Middle Ground (toxic)	
Species	Toxicity (μg STX/100 g)	Density (ind./0.25 m²)	Toxicity (μg STX/100 g)	Density (ind./0.25 m²)
Butter clams		42 ± 13		11 ± 6
Whole	NDT		870	
Siphons only			2,299	
Littleneck clams	NDT	40 ± 11	129	10 ± 4
Horse clams	NDT	5 ± 6	43	13 ± 9
Cockles		3 ± 2	29	2 ± 2

the breeding site and older gulls foraging on clams in a nontoxic area.

METHODS

STUDY AREAS

Tatoosh Island is a breeding site for >4,000 gulls (Paine et al. 1990) and is on the outer coast of Washington State at the mouth of the Strait of Juan de Fuca. The presence of chicks on 29–31 August 1989 allowed me to test the responses of naive birds to PSPT-contaminated prey. In addition, individual birds could be followed during the experimental period because many chicks had not fledged, and family groups remained on their territories.

Scatchet Head, at the south end of Whidbey Island, Washington, and Middle Ground in Sequim Bay, Washington, were selected as nontoxic and toxic sites, respectively. The extensive intertidal gravel flats at both sites are used heavily by gulls to forage on rich populations of cockles (*Clinocardium nuttalli*), littleneck clams (*Protothaca staminea*), butter clams (*Saxidomus giganteus*), and horse clams (*Tresus capax*) (pers. obs.). Prey abundance was sampled using randomly tossed 0.25 m² quadrats at Scatchet Head (n = 5) and Middle Ground (n = 6). All bivalves excavated from the quadrats were identified to species and counted.

The gulls excavate the shallower prey (cockles, littleneck clams, and small butter clams) themselves and obtain deeper clams (horse clams and larger butter clams) from excavations left by clammers. Large numbers of both of these species are routinely uncovered and left exposed by the numerous clammers in search of the preferred littleneck clams at both of these sites (pers. obs.). For this reason it is likely that most, if not all, gulls at both Scatchet Head and Middle Ground have had frequent encounters with all four species of bivalves in this study.

At the time of the study (29 April to 23 May 1990), butter clams at Scatchet Head had not contained detectable levels of PSPT since June 1985 (<37 μg STX equivalents/100 g), or PSPT concentrations above closure level (80 μg STX equivalents/100 g) since September 1983 (Washington State Department of Health unpubl. data). Butter clams at Middle Ground have been chronically well above closure level for >14 yr, generally in the range of 300–1,000 μg STX equivalents/100 g (Washington State Department of Health unpubl. data). Butter clams are the best indicator of site PSPT history because of their extremely long toxin-retention time, whereas the other species mentioned above usually depurate PSPT in 4–11 weeks (Quayle 1969, Shumway 1990). Thus, even though Middle Ground butter clams are dangerously toxic year-round, the cockles, littleneck clams, and horse clams are usually only seasonally toxic for a relatively short time following a "red tide" (Washington State Department of Health unpubl. data).

Toxicity levels were determined for butter clams and littleneck clams collected at both sites, and cockles and horse clams collected at Middle Ground between 26 April and 8 May, 1990. Mouse bioassays (AOAC 1984) were conducted by Washington State Department of Health on all samples except the cockles, which were tested with high performance liquid chromatography (HPLC) (courtesy of M. Beitler, Division of Aquaculture and Food Science, University of Washington, Seattle) by the methods of Sullivan and Wekell (1986) (Table 1).

Gull populations and demographics were also sampled at both sites during the feeding experiments. All individuals in the foraging areas were counted and aged by plumage characteristics (Table 2). Other gull species, if present, were also recorded. Although other species of birds are known to forage on clams, Glaucous-winged Gulls are virtually the only avian residents observed to prey on bivalves at both sites. Only crows are occasionally seen as well (pers. obs.).

FEEDING EXPERIMENTS: GULL CHICKS

Response of naive gull chicks to PSPT-contaminated prey.—Glaucous-winged Gull chicks were tested for

TABLE 2. Density and age composition of Glaucous-winged Gulls at Scatchet Head (nontoxic site) and Middle Ground (toxic site). Populations were not significantly different with respect to population size (t-test, $t = 2.52$, df = 4, $P = 0.09$) or gull age structure ($\chi^2 = 8.19$, df = 1, $P = 0.09$) during the feeding experiment period.

Date	Juveniles (1–2 yr)	Adults (≥3 yr)	n
Scatchet Head (nontoxic site)			
12 May	17%	83%	46
14 May	30%	70%	40
21 May	20%	80%	49
Middle Ground (toxic site)			
13 May	28%	72%	61
23 May	13%	87%	95

their response to toxic prey and their tendency to develop conditioned aversions. Shucked, whole, fresh nontoxic and toxic butter clams were made available to gull chicks on their parents' breeding territories at Tatoosh Island. Nontoxic clams had been collected at Mukilteo, Washington (no detectable toxin, Washington State Department of Health unpubl. data); toxic clams were collected at Middle Ground (445 μg STX equivalents/100 g, Washington State Department of Health unpubl. data). Shucked, rather than intact, prey was used so that the gulls would not have to fly off with the clams to crack them open, which would have made it virtually impossible to keep track of individuals. Butter clams were presented to haphazardly selected family groups by placing the shucked clams within the territory of the adults. The clams were either eaten directly by the chicks, eaten by the adults and immediately (<1 min) regurgitated to the begging chicks, or stolen and eaten by an intruding gull. I observed 88 ingestions (30 nontoxic and 58 toxic) and followed individuals for 15 min or until I observed a regurgitation. All chicks that received nontoxic clams also received toxic clams, but 28 chicks only received toxic clams. The regurgitation of an adult to a chick was not considered to be due to prey toxicity. In addition, 14 of the chicks that ate toxic prey and 11 that ate nontoxic prey were timed individually for 15 min or until regurgitation to determine the time required to detect and regurgitate the toxic clam.

The day following their first exposure to toxic butter clams, I tested seven of the birds for aversion to butter clams. They were offered shucked nontoxic butter clams for 5 min followed by nontoxic horse clams as a control. The sample size was 7 individuals because this was the number that could be unambiguously identified as subjects from the following day.

FEEDING EXPERIMENTS: NONTOXIC VS. TOXIC SITES

The following experiments were designed to determine whether gulls foraging on bivalves at a toxic site had learned to identify and avoid toxic prey. All nontoxic butter clams used as prey were collected at Scatchet Head (hereafter *nontoxic site*) and the toxic ones at Middle Ground (*toxic site*) (Table 1). The other species used in the experiments (cockles, littleneck clams, and horse clams) were locally obtained at each test site (Table 1). Sample sizes are given with the results.

Experiment I: shucked bivalve prey.—The objectives of this experiment were to learn whether (1) free-ranging adult and postfledgling juvenile gulls discriminate between toxic and nontoxic prey of the same and different species, (2) the frequency of toxic prey consumption varies with gull age, (3) gulls selectively eat butter clam tissues, avoiding the highly toxic siphon, (4) older gulls vomit ingested toxic prey as did the chicks on Tatoosh, and (5) gulls at a toxic versus a nontoxic site differ in the above responses. Nontoxic and toxic butter clams and locally obtained horse and littleneck clams were offered to free-ranging gulls of all ages at both the nontoxic site and the toxic site. Shucked clams were again used so that the bird that actually consumed the prey could be more easily identified and followed. Whole, fresh bodies of each species were tossed out in a random series to foraging gulls during low tide. I noted whether each clam was (1) eaten or rejected within 5 min, (2) eaten by an adult (≥3 yr) or juvenile (≤2 yr), (3) entirely eaten or the siphon rejected, (4) entirely eaten and regurgitated within 20 min. Whenever possible, birds that had eaten butter clams were tracked for 20 min, and the time to regurgitation (if it occurred) was noted. I considered 20 min to be a sufficient observation period based on previous feeding experiments. At the nontoxic site this experiment was conducted on an area of beach ca. 200 m away from where the other tests were run, to minimize biasing future tests should the gulls develop PSPT-related aversions to butter clams. This was effective because individual gulls appeared to prefer foraging territories at the site.

Experiment II: three-species predation test.—To determine if there were species-specific differences in bivalve predation rates related to prey toxicities at nontoxic and toxic sites, live intact individual butter clams, cockles, and littleneck clams were made available to foraging gulls at both sites. Groups of three clams each (one individual of each species per group) were laid out at 25–50 m intervals along the beach at low tide while gulls were foraging. The clams were comparable in size and weight, with the butter clam never the largest or smallest in the group. Each group was checked for missing clams after 5–10 min.

Experiment III: prey preference tests.—This experiment was designed to determine if there were age-

and site-specific prey preferences related to prey toxicity patterns. Prey preferences were determined by placing pairs of live intact clams on the beach among foraging gulls at low tide. At Scatchet Head each pair consisted of a locally obtained butter clam matched with a local cockle or littleneck clam of comparable size. At Middle Ground the setup was the same with the addition of horse clams. Each prey pair was laid out and watched individually for 5 min to see which clam species was taken first, if both were taken, and the age of the bird taking each clam. The order of species paired with the butter clam was random. Horse clams were not used at Scatchet Head because they were too large for the gulls to carry away and could not be matched by size with the smaller butter clams.

Statistical Analysis and Assumptions

Unless otherwise stated, I used Chi-square and contingency table analyses to compare the responses of gulls in the experiments, and $P \leq 0.05$ was the significance level for all tests. I assumed that each trial involved a different gull and was thus independent, although this cannot be absolutely assured because none of the birds in the populations were marked. However, because my investigation was focused on population- and community-level effects of sequestered PSPT, testing for differences in the relative frequency of prey species consumption proved a good measure of the differences in gull response to bivalves at the two sites. As a precaution, I minimized the likelihood of testing the same individual by timing and spacing each trial appropriately. Whenever a gull took an intact clam from one of my trials, it generally flew away from the test area and spent several minutes repeatedly flying up and dropping the clam to break it. Also, individuals at both sites had discrete foraging territories along the beach. Thus by restricting the trials to only a few minutes and spacing them more widely than the apparent foraging territory size, and by watching to see that the birds that took clams left the area, I maximized the number of birds tested in each experiment. Feeding trials with shucked clams were also spaced along the beach to maximize the number of birds tested.

Results

Prey Populations at Toxic and Nontoxic Sites

At the nontoxic site, the most abundant bivalve species was the butter clam (47% of all bivalves). At the toxic site, butter clams were only slightly less abundant (31% of all bivalves) than horse clams (36%), the most common clam there (Table 1). None of the bivalves collected at the nontoxic site contained levels of PSPT within the detection range of the mouse bioassay ($\geq$37 μg STX equivalent/100 g, Washington State Department of Health) (Table 1). At the toxic site, all species tested contained some PSPT, but only butter clams and littleneck clams were above State Health Department closure level, and the butter clams were >6 times more toxic than the littleneck clams (Table 1). PSPT levels found in the toxic-site cockles with HPLC were below the detection limit for the mouse bioassay.

Feeding Experiments: Gull Chicks

Response of naive gull chicks to PSPT-contaminated prey.—All gull chicks that ingested shucked toxic butter clams ($n = 58$) regurgitated and abandoned the clam body intact. The mean time ($\pm$SD) to regurgitation was 4.7 $\pm$ 2.1 min ($n = 14$) for chicks fed toxic butter clams, whereas chicks that ingested nontoxic butter clams ($n = 30$) never regurgitated the prey. Of those gulls tested for aversion to butter clams ($n = 7$) the day following their ingestion and regurgitation of toxic butter clams, none would eat available shucked nontoxic butter clams, but all readily consumed shucked horse clams.

Feeding Experiments: Nontoxic vs. Toxic Sites

Gull populations and demographics.—The Glaucous-winged Gull was the dominant gull species observed at both sites during the feeding studies. A single Glaucous-winged × Western (*Larus occidentalis*) gull hybrid was seen once at Scatchet Head. The only other avian predators that I observed were a few (<5) Northwestern Crows (*Corvus caurinus*). Although they were occasionally present at both sites, a crow was observed taking a clam from an experiment only once, and this was omitted from the results. Gull population sizes at Scatchet Head (45 $\pm$ 4.6, $n = 3$) and Middle Ground (78 $\pm$ 24.0, $n = 2$) were not significantly different, nor were the age structures of the two populations (Table 2).

Experiment I: shucked bivalve prey.—Glaucous-winged Gulls foraging at the toxic site, but not at the nontoxic site, displayed a significant aversion to all butter clams and butter-clam siphons, but no aversion to other species (Table 3). Gulls at Middle Ground ate significantly smaller per-

TABLE 3. Response of Glaucous-winged Gulls to shucked, toxic, and nontoxic bivalve prey (Experiment I). In feeding trials at the toxic site, gulls ate significantly fewer shucked butter clams (but not fewer other prey) than at the nontoxic site. The gulls' aversion to butter-clam flesh at Middle Ground was independent of clam toxicity because nontoxic and toxic butter-clam tissues were equally shunned. There was no significant feeding pattern related to gull age in this experiment. Siphons of both nontoxic and toxic butter clams were discarded by gulls in a significant number of trials at the toxic site. Siphons account for the majority of butter-clam toxicity in contaminated individuals. Only toxic butter clams were regurgitated at both sites. NDT = no detectable toxins.

	Shucked, whole bivalve prey available to gulls							
	Butter clam				Horse clam		Littleneck clam	
Gull response	Non-toxic	*n*	Toxic	*n*	Local	*n*	Local	*n*
Scatchet Head (nontoxic site)								
Prey toxicity (μg STX equivalents/100 g)	NDT		870		NDT		NDT	
Total prey eaten (%)	100	20	94	16	100	16	100	15
Prey eaten by adults (% of total prey eaten)	60	20	44	9	44	16	20	15
Siphons rejected (% of total prey eaten)	0	20	0	9	0	16	0	15
Prey regurgitated (% of total clams eaten)	0	20	83	6	—		—	
Time to regurgitation (min, $\bar{x} \pm$ SD)			12.7 ± 6.8	5				
Middle Ground (toxic site)								
Prey toxicity (μg STX equivalents/100 g)	NDT		870		43		129	
Total prey eaten (%)	47	19	55	29	100	16	92	13
Prey eaten by adults (% of total prey eaten)	78	9	44	16	69	16	75	12
Siphons rejected (% of total prey eaten)	89	9	56	16	0	16	0	12
Prey regurgitated (% of total clams eaten)	0	3	75	4	—		—	
Time to regurgitation (min, $\bar{x} \pm$ SD)			14.5 ± 5.0	2				

centages of available shucked toxic and nontoxic butter clams than those of the other available clams (Chi-square tests, $P < 0.05$). They did not, however, appear to distinguish between nontoxic and toxic butter clams ($\chi^2 = 0.055$, df = 1, $P = 0.82$) (Table 3). The percentages of total prey eaten for all available species at the nontoxic site (Scatchet Head) did not differ significantly, nor did gull consumption of nontoxic versus toxic butter clams (Chi-square tests, $P > 0.05$). Gulls at the nontoxic site also ate significantly more butter clams than at the toxic site (Chi-square tests, $P < 0.05$), but there was no difference in the percent consumption of the other two species ($P > 0.05$). There was no obvious or significant pattern in the behavior of adults versus juveniles in this experiment.

Gulls rejected a significant percentage of all butter-clam siphons at the toxic site (Chi-square tests, $P < 0.05$). Siphons of the two other species were never rejected at the toxic site nor were siphons of any of the available prey rejected at the nontoxic site.

Although gulls at the nontoxic site showed no difference in their willingness to eat toxic and nontoxic butter clams, a significant number (83%) of those that ate entire toxic butter clams regurgitated them (Table 3; $\chi^2 = 15.62$, df = 1, $P = 0.0001$). None of the birds that ate the nontoxic butter clams regurgitated during the 20-min observation period. I found the same pattern at the toxic site for the few birds that ate entire butter clams and were followed. A Chi-square test was not significant, perhaps because of the small sample size involved.

Experiment II: three-species predation test.—Gulls at the toxic site avoided live intact butter clams, but not cockles or littleneck clams, when compared with gulls at the nontoxic site (Fig. 1). Gulls at Middle Ground took a significantly lower percentage of both nontoxic and toxic butter clams compared with gulls at the nontoxic site ($\chi^2 = 79.56$, df = 3, $P = 0.0001$). There was no significant between-site difference in the percentages of cockles ($\chi^2 = 2.74$, df = 3, $P = 0.4$) and littleneck clams ($\chi^2 = 2.64$, df = 3, $P = 0.5$) taken by gulls. There were also no within-site differences in the percentages of nontoxic and toxic butter clams taken (Scatchet Head, $\chi^2 = 0.78$, df = 1, $P = 0.38$; Middle Ground, $\chi^2 = 0.50$, df = 1, $P = 0.48$).

Experiment III: prey preference tests.—Gulls took

TABLE 4. Glaucous-winged Gull two-species bivalve prey preference test (Experiment III). In paired prey choice tests at the toxic site (Middle Ground), gulls took significantly fewer butter clams than at the nontoxic foraging site (Scatchet Head). All other bivalve prey paired with butter clams in choice tests (cockles, littleneck clams, and horse clams) were preferred (taken first) more often than butter clams at the toxic site, but not the nontoxic site. Adult gulls at the toxic site seldom took butter clams compared with the other prey available, but took all clams at the nontoxic site as often as juvenile gulls.

	Choice test 1			Choice test 2			Choice test 3			Summary	
Site/gull response	Butter clam	Cockle	*n*	Butter clam	Littleneck clam	*n*	Butter clam	Horse clam	*n*	Butter clam	*n*
Scatchet Head											
% taken	100	100	10	82	82	11				91	21
% taken first	20	80		64	36					43	
% taken by adults	50	70		55	36					52	
Middle Ground											
% taken	50	100	10	66	100	12	29	100	7	52	29
% taken first	10	90		25	75		0	100		14	
% taken by adults	20	100		17	67		14	86		12	

significantly fewer butter clams at the toxic site than at the nontoxic site (all comparisons pooled, $\chi^2 = 7.37$, df = 1, $P = 0.007$; Table 4). The gulls at the nontoxic site showed no significant prey preference in either of the choice tests (Chi-square tests, $P > 0.05$). Middle Ground gulls, however, preferred cockles ($\chi^2 = 6.6$, df = 1, $P = 0.01$) and horse clams ($\chi^2 = 5.25$, df = 1, $P = 0.02$) over butter clams (Table 4). Although littleneck clams were also chosen first more frequently than butter clams at the toxic site, the difference was not significant ($\chi^2 = 3$, df = 1, $P = 0.08$). Of all the butter clams taken at the toxic site, significantly less were taken by adults than at the nontoxic site ($\chi^2 = 5.4$, df = 1, $P = 0.02$).

DISCUSSION

GULL AVOIDANCE OF PSPT

Although paralytic shellfish poisoning toxin has been implicated in several seabird kills (Nisbet 1983, Hockey and Cooper 1980, Coulson et al. 1968, Armstrong et al. 1978, McKernan and Scheffer 1942), my study suggests that Glaucous-winged Gulls are not at mortal risk from bivalve-borne PSPT. None of the gulls fed toxic butter clams died or showed symptoms displayed by other birds that became ill or died following the ingestion of contaminated butter clams (Kvitek and Beitler 1988). The absence of illness in the gulls was probably not due to insufficient prey toxicity; rather, inexperienced birds regurgitated the toxic prey within a few minutes after ingestion (<4 min for Tatoosh chicks, and <15 min for all postfledglings and adults; Table 3). Thus the toxin was voided before serious illness occurred.

Gulls appear to be protected further from PSPT via food aversion. This hypothesis is supported by two lines of evidence. First, all of the seven chicks on Tatoosh followed as individuals for 2 days appeared to have developed a conditioned aversion to nontoxic butter-clam tissue—but not to horse clams—on the day after their first ingestion and regurgitation of toxic butter clams. Second, gulls at the site where butter clams are chronically toxic generally avoided butter clams independent of toxicity (Table 3, Fig. 1). I believe that once an inexperienced gull eats and regurgitates a toxic individual, it will reject that prey species on the next encounter. The aversion thus appears to be generalized to the prey species and not to the toxicity of prey individuals encountered after conditioning. My results, however, do not preclude the possibility of socially transmitted, PSPT-mediated prey aversions within a gull population. Because butter clams but not other species at Middle Ground have been chronically toxic year-round for many years, it is conceivable that a tradition of butter-clam avoidance has arisen and been transmitted from parent to offspring at that site. Such avoidance behavior would be further reinforced any time a gull ate a butter clam at Middle Ground, because although toxicity may vary between individual butter clams, virtually all butter clams

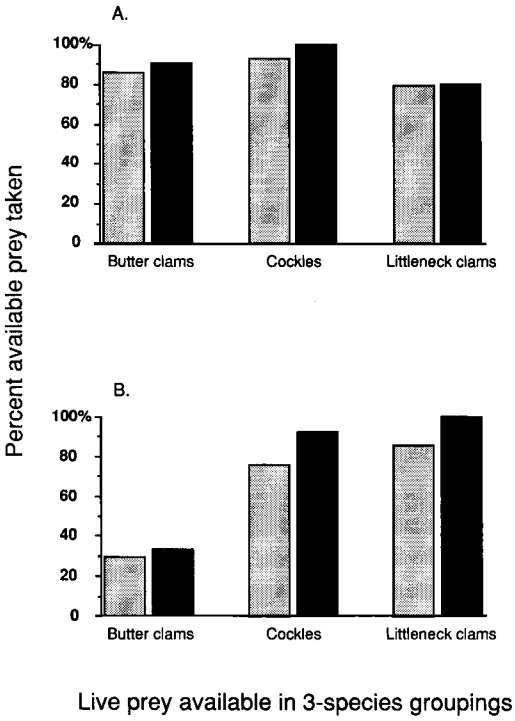

Fig. 1. Percentages of the available live bivalve prey "taken" by Glaucous-winged Gulls at Scatchet Head (A: nontoxic site) and Middle Ground (B: toxic site). Stippled bar represents prey grouped with nontoxic butter clams (n = 28 groups at Scatchet Head and 21 groups at Middle Ground) and solid bar represents prey grouped with toxic butter clams (n = 10 and 24). Both nontoxic and toxic butter clams were taken significantly less frequently at Middle Ground, the toxic site (χ^2 = 79.56, df = 3, P = 0.0001).

will contain biologically significant amounts of PSPT (Quayle 1969). The tendency for juvenile gulls to choose intact butter clams more often than adult gulls do (Table 4) implies that differences in either experience level or age-related learning ability (Evans et al. 1987) may be important in the development of prey aversion in gulls.

Aversive behavior toward food in birds has been associated with neophobia, the fear of novel food (Evans et al. 1987). This is an unlikely explanation for the response of the gulls toward butter clams at Middle Ground. Butter clams are abundant, and second only to the horse clam in abundance at Middle Ground (Table 1). Although large butter clams are deeper burrowers than cockles or littleneck clams, smaller individuals are found at comparable depths and have been reported as important in the diets of some Glaucous-winged Gull populations (Barash et al. 1975, Vermeer 1982). Furthermore, gulls at Middle Ground gain access to even the largest butter clams because this species is excavated and left exposed on the beach in great numbers by clammers looking for the more preferred (less toxic) littleneck. Thus, the high abundance of butter clams (Table 1), the shallow burrow depth of smaller individuals, and the activity of clammers insures that most gulls at Middle Ground undoubtedly have encountered butter clams. Indeed, if neophobia were an important factor in the aversive behavior of sea gulls toward any clam at Middle Ground, I would expect it to be most pronounced toward the horse clam. This species is a deeper burrower, even at small sizes, than the butter clam, and thus it is encountered much less frequently by gulls excavating their own prey. Furthermore, horse clams are morphologically the most distinct of the four species (Morris et al. 1980). Nevertheless, horse clams at Middle Ground were readily accepted as prey by the gulls, whereas butter clams, although very similar in appearance to littleneck clams, were generally avoided (Tables 3 and 4).

Gull aversion to PSPT-contaminated prey resembles closely the behavior of insectivorous birds that shun unpalatable insects as well as their nontoxic mimics and conspecifics (Brower and Glazier 1975, Brower and Fink 1985). Unlike birds known to discriminate between toxic and nontoxic individuals (Calvert et al. 1979, Brower and Fink 1985), Middle Ground gulls generally shunned nontoxic as well as toxic butter clams (Tables 3 and 4, Fig. 1). Some of the gulls at the toxic site ate butter clams, but they generally discarded the siphon (the organ which becomes most toxic in contaminated butter clams, Quayle 1969) (Tables 1, 3, and 4). This behavior also occurs in sea otters (Kvitek et al. in press) and is similar to that of insectivorous birds that selectively eat only the least toxic parts of their prey (Brown and Neto 1976, Fink and Brower 1981, Brower and Fink 1985). It is not clear how gulls learn to avoid the siphons, particularly since they do not appear to distinguish between toxic and nontoxic butter-clam tissues (Table 3). Once acquired, this behavior could conceivably be socially transmitted (Galef 1987). Additional work will be required to determine how the initial learning takes place.

PSPT and Seabird Mortalities

Some seabirds die from PSPT-laden prey (McKernan and Scheffer 1942, Coulson et al. 1968, Bicknell and Walsh 1975, Sasner et al. 1975, Armstrong et al. 1978, Hockey and Cooper 1980, Nisbet 1983), while others, such as the Glaucous-winged Gull, appear to be behaviorally exempt from risk. Rather than being species-specific, differential susceptibility may well be mediated via prey type. Most seabird kills attributed to PSPT involved planktivorous fish rather than bivalves as vectors. If seabirds consume small fish intact, without rupturing the body wall, PSPT confined to the fish gut may not be released and detected until much later in the digestion process and perhaps too late to regurgitate and avoid absorption. In contrast, birds break the shell of bivalve prey and consume the viscera directly. Therefore, even predators that lack chemosensory receptors for PSPT may still be able to detect the early symptoms of PSP shortly after the ingestion of toxic bivalves, when it is still possible to regurgitate the prey intact. The gull chicks at Tatoosh were exceptionally sensitive to ingested PSPT-laden butter clams, and they regurgitated them in <5 min, more rapidly than required for PSP symptoms to be detected in humans (Halstead 1978) or for observable symptoms to appear in starlings (Kvitek and Beitler 1988). Indeed, it is possible that Glaucous-winged Gulls have become "physiologically tuned" to PSPT to a greater extent than other birds and are thus better able to detect and avoid a lethal dose.

Seabird susceptibility to PSPT may also be mediated by novelty and toxin level. The only reported massive mortality of seabirds associated with PSPT-laden bivalves involved extraordinarily toxic shellfish (9,500 μg STX equivalents/100 g) in a New England area previously free of PSPT (Sasner et al. 1975). There is also an anecdotal report of resident avian bivalve predators shunning toxic shellfish that killed migrating Black Ducks (Tufts 1979). These accounts are consistent with my results. Naive chicks at Tatoosh and gulls at the nontoxic site readily consumed toxic butter clams when first encountered. Perhaps if these clams had been sufficiently toxic, the birds may not have been able to regurgitate their food in time to avoid a lethal dose. Thus, birds foraging at a site that is frequently toxic may have the advantage of learning which prey to avoid when bivalves contain only moderate amounts of residual toxin.

PSPT and Predator/Prey Relationships

Paralytic shellfish poisoning toxin may not pose a significant threat to most avian predators of bivalves, but it does appear to profoundly affect Glaucous-winged Gull prey selection. The experienced gulls in this study exhibited a marked aversion to toxic species. As a result, gulls took significantly fewer butter clams at a chronically toxic site than inexperienced gulls at a nontoxic site (Fig. 1, Table 4). Yet there was no difference between sites in the frequency with which gulls took other nontoxic or low-toxicity prey. Because the aversion at the toxic site was species-specific and independent of prey toxicity, avian predation pressure on bivalve species that sequester PSPT may be reduced both beyond the period of time that the clams remain dangerously toxic and perhaps at nontoxic sites at which conditioned birds also forage.

Superficially, it appears that some bivalves may sequester PSPT, which acts as a chemical deterrent to avian predation and is analogous to the milkweed–monarch butterfly–Blue Jay relationship (Brower and Fink 1985, but see Brower et al. 1988). Gulls, however, always kill the individual bivalve. It is thus difficult to imagine how PSPT retention may be selected by avian predation without invoking group selection. Furthermore, unlike most of the birds known to avoid cardenolide-laden butterflies, the gulls in this study did not reject their prey before consumption, based on the presence of toxin (Fig. 1).

A more likely selective agent for the retention of PSPT in bivalves would be "grazing" predators, such as siphon-cropping fish (Kvitek and Beitler 1991), which do not kill their prey but do develop a PSPT-based aversion to toxic but not nontoxic butter-clam siphons (Kvitek MS). Siphon-cropping fish have been linked with significant reductions in bivalve growth rates (Peterson and Quammen 1982, Zwarts 1986), presumably because resources must be shunted from growth and reproduction to siphon regeneration. However, retention of PSPT in the butter-clam siphon may have been indirectly selected for by avian predation, because a fish nipping off the tip of a siphon causes the clam to reduce its burrow depth and this in-

creases its vulnerability to bird predation (Zwarts 1986). Thus, a defense strategy that reduces partial predation by fish will permit the clam to retain its burrow depth and refuge from birds.

Another reason avian predation may not be the most important mechanism selecting for PSPT retention in butter clams is that burrow depth increases with bivalve size (pers. obs.). Larger individuals generally have a burrow depth (10–30 cm, Morris et al. 1980, pers. obs.) that is below the foraging range of most birds (Roberts et al. 1989, but see Vermeer and Bourne 1984). Although gulls are frequently observed eating large butter clams, this is most common on beaches where clam diggers are excavating and discarding or overlooking butter clams, or where burrow depth is restricted because of underlying clay or rock (pers. obs.). The most frequently observed bivalve prey species that gulls and crows excavate unaided are cockles and littleneck clams, which are usually just below the sediment surface (Oldham 1930, Barash et al. 1975, Roberts et al. 1989, Richardson and Verbeek 1986, pers. obs.). Both of these species have relatively short PSPT retention times (9 and 5 weeks, respectively) compared with butter clams (>1 yr) (Shumway 1990). Furthermore, cockles and littleneck clams at Middle Ground, the site at which butter clams are chronically very toxic, were far less toxic than the butter clams and were readily consumed by the gulls there (Tables 1 and 3, Fig. 1). Therefore the value of PSPT retention in butter clams as a deterrent to avian predation may be most important only to smaller size classes or ancillary to its role as a defensive agent against fish. Yet the extreme lethality and range of taxa susceptible to PSPT (Halstead 1978) may extend the usefulness of these toxins as a chemical defense once the ability to sequester them has evolved. For example, the retention of PSPT may profoundly influence predation by scoters (*Melanitta* spp.), for which butter clams make up the majority of prey at some winter sites (Vermeer and Bourne 1984).

Avian predators of bivalves may be at a much lower risk to PSPT than piscivorous birds because of the tendency of the former to regurgitate rapidly the ingested PSPT-laden prey. The risk of poisoning is further reduced via a conditioned avoidance of previously regurgitated bivalve species. For this reason, PSPT may function as an effective deterrent to avian predation on bivalves that sequester the toxin. This aversion appears to be independent of the prey's current level of toxicity, and the protection it affords may be extended in time and space beyond both the toxin-retention period and geographic range of prey contamination. Although experienced gulls in this study avoided butter clams, siphon-nipping fish are a more likely selective mechanism for the evolution of long-term PSPT retention in *Saxidomus*. Nevertheless, once evolved, the broad spectrum of taxa susceptible to PSPT widens its effectiveness as an acquired chemical defense wherever toxic blooms of *Protogonyaulax* spp. occur.

ACKNOWLEDGMENTS

I am very grateful to D. Carney, R. T. Paine, C. Pfister, and T. Wootton for field assistance and for suggestions that substantially improved this manuscript. I am also indebted to M. Beitler and G. Skow for PSPT analyses without which this work would not have been possible. Special thanks go to R. T. Paine, who has generously supported this project through a National Science Foundation award (OCE 8614463). Additional support was provided by a NSF award to J. Oliver (DPP-8619394), and National Audubon Graduate Student Research and American Museum of Natural History Lerner-Gray Fund for Marine Research awards to R. Kvitek. Research on Tatoosh was made possible by permission of the Makah Tribal Council and the U.S. Coast Guard.

LITERATURE CITED

ARMSTRONG, I. H., J. C. COULSON, P. HAWKEY, & M. J. HUDSON. 1978. Further mass seabird deaths from paralytic shellfish poisoning. Br. Birds 71: 58–68.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. Official methods of analysis (S. Williams, Ed.). Arlington, Virginia, Assoc. Offic. Anal. Chem.

BARASH, D. P., P. DONOVAN, & R. MYRICK. 1975. Clam dropping behavior of the Glaucous-winged Gull (*Larus glaucescens*). Wilson Bull. 87: 60–64.

BICKNELL, W. J., & D. C. WALSH. 1975. The first "red tide" in recorded Massachusetts history. Pp. 447–457 *in* Proceedings of the First International Conference on Toxic Dinoflagellate Blooms (V. R. LoCicero, Ed.). Wakefield, Massachusetts, Massachusetts Sci. Tech. Found.

BOURNE, N. 1984. Clam predation by scoter ducks in the Straits of Georgia, British Columbia, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 1331.

BROWER, L. P., & L. S. FINK. 1985. A natural toxic defense system: cardenolides in butterflies versus birds. Ann. N.Y. Acad. Sci. 443: 171–188.

———, & S. C. GLAZIER. 1975. Localization of heart poisons in the monarch butterfly. Science 188: 19-25.

———, C. J. NELSON, J. N. SEIBER, L. S. FINK, & C. BOND. 1988. Exaptation as an alternative to coevolution in the cardenolide-based chemical defense of monarch butterflies (*Danaus plexippus* L.) against avian predators. Pp. 447-475 *in* Chemical mediation of coevolution (K. Spencer, Ed.). San Diego, Academic Press.

BROWN, K. S., & J. V. NETO. 1976. Predation on aposematic ithomiine butterflies by tanagers (*Pipraeidea melanonota*). Biotropica 8: 136-141.

CALVERT, W., L. E. HEDRICK, & L. P. BROWER. 1979. Mortality of the monarch butterfly (*Danaus plexippus* L.): avian predation at five overwinter sites in Mexico. Science 204: 847-851.

COULSON, J. C., G. R. POTTS, I. R. DEANS, & S. M. FRASER. 1968. Exceptional mortality of shags and other seabirds caused by paralytic shellfish poison. Br. Birds 61: 381-405.

EVANS, D. L., N. CASTORIADES, & H. BADRUDDINE. 1987. The degree of mutual resemblance and its effect on predation in young birds. Ethology 74: 335-345.

EVANS, P. R., D. M. HERDSON, P. J. KNIGHT, & M. W. PIENKOWSKI. 1979. Short-term effects of reclamation of part of seal sands, Teesmouth, on wintering waders and shelducks. I. Shorebird diets, invertebrate densities, and the impact of predation on the invertebrates. Oecologia 41: 183-206.

FINK, L. S., & L. P. BROWER. 1981. Birds can overcome the cardenolide defence of the monarch butterflies in Mexico. Nature 291: 67-70.

GALEF, B. G., JR. 1987. Social influences on the identification of toxic foods by Norway rats. Anim. Learn. and Behav. 15: 327-332.

HALSTEAD, B. W. 1978. Poisonous and venomous marine animals of the world. Princeton, Darwin Press.

HOCKEY, P. A. R., & J. COOPER. 1980. Paralytic shellfish poisoning—a controlling factor in Black Oystercatcher populations? Ostrich 51: 188-190.

KVITEK, R. G., & M. K. BEITLER. 1988. A case for sequestering of paralytic shellfish toxins as a chemical defense. J. Shellfish Res. 7: 629-636.

———, & ———. 1989. An addendum to a case for sequestering of paralytic shellfish toxins as a chemical defense. J. Shellfish Res. 8: 253.

———, & ———. 1991. Relative insensitivity of butter clam neurons to saxitoxin: an exaptation for sequestering paralytic shellfish poisoning toxins as a chemical defense. Mar. Ecol. Prog. Ser. In press.

———, A. R. DEGANGE, & M. K. BEITLER. In press. Paralytic shellfish poisoning toxins mediate sea otter feeding behavior. Limnol. Ocean.

MCKERNAN, D. L., & V. B. SCHEFFER. 1942. Unusual numbers of dead seabirds on the Washington coast. Condor 44: 264-266.

MORRIS, R. H., D. P. ABBOTT, & E. C. HADERLIE. 1980. Intertidal invertebrates of California. Stanford, California, Stanford Univ. Press.

NETTLESHIP, D. N., G. A. SANGER, & P. F. SPRINGER. 1984. Marine birds: their feeding ecology and commercial fisheries relationships. Seattle, Washington, Annual Meeting of the Pacific Seabird Group.

NISBET, I. C. 1983. Paralytic shellfish poisoning: effects on breeding terns. Condor 85: 338-345.

NISHITANI, L., & K. CHEW. 1988. PSP toxins in the Pacific coast states: monitoring programs and effects on bivalve industries. J. Shellfish Res. 7: 653-669.

O'CONNOR, R. J., & R. A. BROWN. 1977. Prey depletion and foraging strategy in the Oystercatcher *Haematopus ostralegus*. Oecologia 27: 75-92.

OLDHAM, C. 1930. The shell-smashing habit of gulls. Ibis 6: 239-242.

PAINE, R. T., J. T. WOOTTON, & P. D. BOERSMA. 1990. Direct and indirect effects of Peregrine Falcon predation on seabird abundance. Auk 107: 1-9.

PETERSON, C. H., & M. L. QUAMMEN. 1982. Siphon nipping: its importance to small fishes and its impact on growth of the bivalve *Protothaca staminea* (Conrad). J. Exp. Mar. Biol. Ecol. 63: 249-268.

PRAKASH, A., J. C. MEDCOF, & A. D. TENNANT. 1971. Paralytic shellfish poisoning in eastern Canada. Fish. Res. Bd. Can. Bull. 177: 1-88.

QUAMMEN, M. L. 1984. Predation by shorebirds, fish, and crabs on invertebrates in intertidal mudflats: an experimental test. Ecology 65: 529-537.

QUAYLE, D. B. 1969. Paralytic shellfish poisoning in British Columbia. Ottawa, Canada, Fish. Res. Bd. Can. Bull. 168.

RECHER, H. F. 1966. Some aspects of the ecology of migrant shorebirds. Ecology 47: 393-406.

RICHARDSON, H., & N. A. M. VERBEEK. 1986. Diet selection and optimization by Northwestern Crows feeding on Japanese littleneck clams. Ecology 67: 1219-1226.

ROBERTS, D., D. RITTSCHOF, D. J. GERHART, A. R. SCHMIDT, & L. G. HILL. 1989. Vertical migration of the clam *Mercenaria mercenaria* (L.) (Mollusca: Bivalvia): environmental correlates and ecological significance. J. Exp. Mar. Biol. Ecol. 126: 271-280.

SANGER, G. A., & R. D. JONES. 1982. The winter feeding ecology and trophic relationships of marine birds in Kachemak Bay, Alaska. Final Reports of Principal Investigators, Environmental Assessment of the Alaskan Continental Shelf, NOAA.

SASNER, J. J., JR., M. IKAWA, & B. E. BARRETT. 1975. The 1972 red tide in New Hampshire. Pp. 517-523 *in* Proceedings of the First International Conference on Toxic Dinoflagellate Blooms (V. R.

LoCicero, Ed.). Wakefield, Massachusetts, Massachusetts Sci. Tech. Foundation.

SHUMWAY, S. E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. J. World Aquaculture Soc. 21: 65–104.

SULLIVAN, J. J., & M. M. WEKELL. 1986. The application of high performance liquid chromatography in a paralytic shellfish poisoning monitoring program. Pp. 357–371 *in* Seafood quality determination (D. E. Kramer and J. Liston, Eds.). New York, Elsevier.

TUFTS, N. R. 1979. Molluscan transvectors of paralytic shellfish poisoning. Pp. 403–408 *in* Toxic dinoflagellate blooms (D. L. Taylor and H. H. Seliver, Eds.). New York, Elsevier/North-Holland.

VERMEER, K. 1982. Comparison of the diet of the Glaucous-winged Gull on the east and west coasts of Vancouver Island. Murrelet 63: 80–85.

———, & N. BOURNE. 1984. The White-winged Scoter diet in British Columbia waters: resource partitioning with other scoters. Pp. 30–38 *in* Marine birds: their feeding ecology and commercial fisheries relationships (D. N. Nettleship, G. A. Sanger, and P. F. Springer, Eds.). Seattle, Washington, Annual Meeting of the Pacific Seabird Group.

WHITE, A. W. 1977. Dinoflagellate blooms as probable cause of an Atlantic herring (*Clupea harengus harengus*) kill, and pteropods as apparent vector. J. Fish. Res. Board Can. 34: 2421–2424.

———. 1980. Recurrence of kills of Atlantic herring (*Clupea harengus harengus*) caused by dinoflagellate toxins transferred through herbivorous zooplankton. J. Can. Fish. Aqua. Sci. 37: 2262–2265.

———. 1981a. Sensitivity of marine fishes to toxins from the red-tide dinoflagellate *Gonyaulax excavata* and implications for fish kills. Mar. Biol. 65: 255–260.

———. 1981b. Marine zooplankton can accumulate and retain dinoflagellate toxins and cause fish kills. Limnol. Ocean. 26: 103–109.

ZWARTS, L. 1986. Burying depth of the benthic bivalve *Scorbiularia plana* (da Costa) in relation to siphon-cropping. J. Exp. Mar. Biol. Ecol. 101: 25–39.