

## DOMOIC ACID UPTAKE AND DEPURATION IN DUNGENESS CRAB (*CANCER MAGISTER DANA 1852*)

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**ABSTRACT** The potent marine neurotoxin domoic acid (DA) was detected in razor clams and Dungeness crabs on the Pacific Coast of the United States in 1991, resulting in temporary closures of these fisheries. Closures protect the health of human consumers of clams and crabs but impose significant economic losses to the communities that are dependent on these fisheries. Widespread closures, and in the case of the clams long-lasting ones, were necessary risk management strategies because our knowledge of DA uptake and movement through the food web is very limited. In order to resolve some of these issues and provide health managers with better information concerning this toxin, experiments were conducted on the accumulation and fate of DA in Dungeness crabs. Such information could provide enhanced safety, permit more efficient closures, and lessen the economic effect of future outbreaks.

In the first study, razor clams, containing known concentrations of DA, were fed to Dungeness crabs for 5 days to determine the uptake of the toxin by the crabs. Twenty-four hours after the crabs ingested an initial 960 µg of toxin, 260 µg of DA (27%) was found in the hepatopancreas (HP) of the crabs. At the end of 6 days, 68% (2,850 µg), from an accumulated 4,220 µg of ingested toxin, was present in the HP. DA was never found in the hemolymph or edible muscle of crabs in this experiment, but DA was found in the feces, indicating a route of depuration.

The second study examined the depuration of DA by crabs under fed and starved conditions. Crabs fed DA-contaminated clams for 4 days achieved an average concentration of 69.5 µg of DA/g of HP. After 7 days, crabs that were fed toxin-free clams three times per week showed a 38% reduction in DA concentration, to 43.4 µg of DA/g, whereas the average toxin concentration in the HP of crabs that were starved was reduced by only 4%, to 66.9 µg of DA/g.

In the last sampling, taken at 21 days, the concentration of DA in the HP of fed crabs decreased by 89% of the initial DA concentration to 7.6 µg of DA/g, but that of the starved crabs decreased by only 57%, to 29.7 µg of DA/g. Differences in mean concentrations between starved and fed crabs at 7, 14, and 21 days were significant. Additional measurements at 21 days showed the average weight of a starved crab's HP was only 53% of the fed crab's HP (25.7 vs. 48.7 g). Although the mean weight of the starved crabs (770 g) was greater than that of the fed crabs (730 g), the difference was not significant.

**KEY WORDS:** Domoic acid, Dungeness crab, *Cancer magister*, razor clams, *Siliqua patula*, amnesic shellfish poisoning

### INTRODUCTION

The Dungeness crab fishery of the Pacific Coast of the continental United States and Alaska is an important and well-established industry that supports a large domestic market as well as overseas markets. During the 1994 commercial season, 46 million pounds of Dungeness crabs (*Cancer magister*) were landed, with an exvessel value of over 63 million dollars (Anonymous 1995). There is also a large recreational fishery for Dungeness crab on the Pacific Coast that contributes to an important tourist industry. Between 1981 and 1991, an estimated 683,400 pounds of crab per year were caught recreationally in Washington alone (J. Odell, WA State Department of Fish & Wildlife, pers. comm. 1996).

In the fall of 1991, the Pacific Coast Dungeness crab fishery was involved in an outbreak of domoic acid (DA) poisoning when the toxin was found in the internal organs of the crab. The presence of high concentrations of the toxin in crab viscera resulted in a

temporary closure of the fishery. The closure had a significant economic effect on the industry and communities directly and indirectly connected to the harvest of crabs.

A potent neuroexcitatory amino acid, DA is a naturally occurring marine toxin that contaminated not only Dungeness crabs that year, but other marine species as well as seabirds. Although a diatom of the genus *Nitzschia* was suspected as the cause of the outbreak, it was never confirmed. Four marine phytoplankton species of the genus *Pseudo-nitzschia* [*P. multiseries* (Hasle) Hasle, *P. delicatissima* (Cleve) Heiden, *P. australis* Frenguelli, and *P. seriata* (Cleve) Peragallo] are known producers of DA (Bates et al. 1989, Martin et al. 1990, Garrison et al. 1992, Work et al. 1993, Lundholm et al. 1994, Hasle 1995).

The consumption of DA-contaminated food by humans can cause mild to severe gastrointestinal illnesses and/or neurological symptoms such as disorientation and memory impairment. Although in high doses, DA can be fatal to anyone, the elderly and

health-compromised individuals are especially vulnerable. Because one of the symptoms of the poisoning can be varying degrees of loss of short-term memory (Perl et al. 1990a, Perl et al. 1990b, Zatorre 1990), and the vector was blue mussels, the DA intoxication was named amnesic shellfish poisoning. Stewart et al. (1990) considered DA intoxication symptoms more characteristic of dementia; nevertheless, it should be referred to by the more accurate name, DA poisoning (DAP), because fin fish are also a vector for the toxin.

To date, only two known outbreaks of DAP have been reported, both occurring on the North American continent. The first occurred in 1987 and resulted from human consumption of DA-contaminated, commercially cultivated, blue mussels (*Mytilus edulis* Linnaeus) from Prince Edward Island, Canada. These mussels were later shown to have fed on toxic phytoplankton, *P. multiseptatus* (Bates et al. 1989, Wright et al. 1989). Over 100 people suffered ill effects brought on by the toxin, and three deaths were attributed to DAP.

The second outbreak of DAP occurred in 1991 in Monterey Bay, CA, and involved brown pelicans (*Pelecanus occidentalis* Linnaeus) and Brandt's cormorants (*Phalacrocorax penicillatus* Brandt). These seabirds consumed anchovies (*Engraulis mordax* Girard) that had fed on DA-producing phytoplankton, *P. australis* (Fritz et al. 1992, Work et al. 1993). Shortly after the toxin's presence was noted in California, it was also detected in marine animals from the coastal waters of Washington and Oregon, where razor clams (*Siliqua patula* Dixon) and Dungeness crabs (*C. magister* Dana) were found contaminated with DA. Because of quick action by state and federal agencies and support from the Canadian scientists involved in the 1987 occurrence, no human seafood illnesses from DA were confirmed during this outbreak. As a result, the toxin's effect on the Pacific Coast of the United States was primarily economic, with the closures of the Dungeness crab and razor clam fisheries heavily affecting fishermen, processors, and local economies of the coastal communities dependent on those fisheries.

The initial testing of crabs for the presence of DA during the outbreak indicated that the toxin was confined to the viscera. Continued research has clearly demonstrated that DA is found only in the digestive system of Dungeness crab, primarily in the hepatopancreas (HP) (Wekell et al. 1994a, Lund 1995). Similarly, lobsters (*Homarus americanus*) are known to become toxic with paralytic shellfish poison (PSP), which like DA, contaminates the digestive gland (HP). The problem is significant enough that the Canadian government included lobster in its PSP-monitoring program (Watson-Wright et al. 1991, Lawrence et al. 1994). The presence of DA can pose a health risk to people who eat the HP or "crab butter." For public safety, the action level for the toxin in crab viscera was set at 30 µg of DA/g of viscera (U.S. Food and Drug Administration [FDA] 1993).

DA can also cause economic problems for the industry, especially with respect to crabs earmarked for whole cooked product markets. The presence of the toxin could require that crabs be butchered, cleaned, and cooked before being placed on the market, thus reducing profit margins or in some cases causing loss of market. More information about the fate of DA in Dungeness crabs, therefore, was needed not only by public health officials, but also by the industry to improve its ability to maintain a viable market.

The goal of this study was to provide information about the uptake and depuration of DA in Dungeness crabs to answer the

following questions: How rapid is DA uptake? Does ingested DA accumulate in crab tissues? Will crabs depurate accumulated DA? Do conditions of starvation or feeding affect the depuration of DA from crabs?

## MATERIALS AND METHODS

### Crabs

Two experiments were conducted. In the first experiment, Dungeness crabs were fed DA-contaminated clam meat to monitor the accumulation of DA in their HP. The second experiment monitored the depuration of DA from DA-contaminated crabs under starved and fed conditions.

Live Dungeness crabs used for these experiments were purchased from local commercial wholesalers. Fifty-two crabs were used in the first experiment, and 48 were used in the second experiment. Before their use in the experiments, each lot of crabs was randomly sampled and analyzed for DA to ensure that they were free of the toxin (<0.5 µg of DA/g of HP, as determined by high-performance liquid chromatographic [HPLC] analysis).

The weight of crabs in these experiments averaged 750 g (SD, ±84). They were marked with an identifying number and placed in live holding tanks that were supplied with gravel-filtered, flow-through seawater (~12 L/min). Dissolved oxygen levels in the seawater system ranged between 8.6 and 9.0 ppm, and water temperatures were between 12 and 13°C during the experiments.

### Clams

Razor clams from the 1991 harvest, naturally contaminated with DA, were used to feed the crabs in these experiments. Purchased from a commercial source, the clams had been cleaned, canned, and frozen during the 1991 season, before the awareness of the DA outbreak and the discovery that DA concentrations in clams exceeded the FDA action level of 20 µg of DA/g of wet tissue (U.S. FDA 1993). The clams were subsequently removed from the market. Toxin levels in the clam tissues used in feeding the crabs ranged between 26.2 and 124.6 µg of DA/g. Commercially processed razor clam meats from Alaska, free of any detectable DA (<0.5 µg/g) were used for feeding crabs during acclimation to the laboratory environment and for the depuration study.

### Feeding Crabs

Before being fed DA-contaminated clams, each lot of crabs was acclimated to its new environment for at least 24 h. In preparing the clams as feed, the product was first blotted with paper towels to remove excess thaw drip and then cut into 2- to 5-g pieces. Ten to 15 g of the clam pieces was weighed into individual trays, with each tray receiving the same weight of one or more clam parts (foot, body/mantle, or siphon).

A hand-feeding method, specifically developed for this work, isolated each crab in a net for individual feeding. Feeding was aided by the use of mechanical fingers, i.e., a spring-loaded extension tool (Lund 1995). Identification numbers on crabs made it possible to ensure that all crabs were fed once and only once at each feeding. Estimates of how much DA (µg) was consumed at each feeding were made by multiplying the weight of the clam meat portion fed to the crabs by the concentration of DA in a representative sample of the day's feed. The percentage of DA present in the HP 24 h postprandial was determined from the

product of the weight of the HP and its DA concentration, and dividing by the product of the weight of razor clam meat fed and its DA concentration, that is, DA-HP burden divided by DA-feed burden.

#### *Sample Preparation and Storage*

At each sampling, six to eight crabs were randomly selected from the holding tanks for dissection. All sample lots consisted of crabs taken 24 h postprandial, except for crabs sampled at 4 and 11 h in the uptake experiment. Before dissection, the crabs were drained, weighed, and pithed. The carapace was removed by cutting around its perimeter, and the epidermis covering the viscera was removed to expose the HP tissue. In the uptake experiment, all of the HP tissue was removed and weighed. In the depuration experiment, a subsample (ca. 10–22 g) consisting of HP material removed from the dorsal and ventral areas of the body cavity and lateral spaces under the carapace of each crab was composited and weighed.

To facilitate sampling procedures in experiment 1, some crabs were frozen whole at  $-10^{\circ}\text{C}$  on arrival at the laboratory. When examined, the crabs were partially thawed to prevent excessive leaching, and the HP was sampled as described above and analyzed for DA. In Experiment 2, tissue samples were stored at  $1^{\circ}\text{C}$  until analyzed, usually within 24 h, or if necessary, frozen until analyzed. Drip was considered part of the sample and was mixed in before subsampling for analysis.

#### *Analysis*

DA concentration in the tissues was determined by the HPLC method of Quilliam et al. (1989, 1991), as modified by Halford et al. (1994), in which DA was extracted from HP tissues with an aqueous methanol solution and then purified and eluted through a strong anion solid-phase extraction cartridge with saline-acetonitrile solutions. Samples were run isocratically at  $40^{\circ}\text{C}$  with a reverse-phase C18 column at a flow rate of 0.300 mL/min. The mobile phase was water, acetonitrile, and trifluoroacetic acid (90/10/0.1, v/v/v). A photodiode array detector was set at 242 nm.

### EXPERIMENTAL

#### *Experiment 1—Uptake of DA*

The purpose of this experiment was to determine the uptake, that is, the amount of toxin present in the HP after feeding Dungeness crabs DA-contaminated clam meat. After the first feeding of toxic clam meat (Time 0), the crabs were sampled at 4, 11, and 24 h to determine the time for the toxin to penetrate the HP. Thereafter, the crabs were fed every 24 h for a total of five feedings, i.e., 0, 24, 48, 72, and 96 h. Because sampling procedures followed each feeding by 24 h, the last crabs, fed at 96 h, were sampled at 120 h.

In this experiment, the DA burden of a crab was determined by multiplying the concentration of DA in sampled tissue by the total weight of the crab's HP material. The DA-HP burden is the total amount ( $\mu\text{g}$ ) of toxin in the HP of a crab.

#### *Experiment 2—Depuration of DA*

The purpose of this experiment was to determine the effect of feeding on the depuration rate of DA from crabs. To get crabs to

an arbitrary toxin level over  $50\text{ }\mu\text{g}$  of DA/g of HP, they were fed DA-contaminated clam meat each day for 4 days. Each crab was fed an average of 12.5 g/day of toxic clam meat for a total of 50 g containing  $6,000\text{ }\mu\text{g}$  of DA. Twenty-four hours after the final toxic feeding, eight crabs were sacrificed and analyzed to determine the starting concentration of DA for the depuration experiment. At this time, the remaining crabs were evenly divided into the two treatment groups (fed and starved). Crabs in the fed group received from 5–7 g of DA toxin-free razor clam meat, three times per week for 3 wk. Random samplings of crabs from each group were taken at 7, 14, and 21 days, and HP samples were removed for DA analysis. In addition, the remaining HP tissues from all of the crabs sampled at Day 21 were removed, composited for each treatment group, and weighed and an estimated mean HP weight of a starved and a fed crab was determined.

Hemolymph samples from crabs in both treatment groups were obtained by severing a leg at the coxa and collecting the drip from five crabs at Day 1, i.e., 1 day after the last toxic feeding, and at Day 21. Fecal matter was gathered at the time of sacrifice, by extruding it from the distal portion of the hindgut. Fecal samples in both experiments were composited from three to eight crabs at each sampling and analyzed for DA as described above.

#### *Statistics*

Mean differences between treatments at 7, 14, and 21 days were tested by Student *t*-test using Statview (Abacus Concepts Inc., Berkeley, CA, 1992).

### RESULTS

#### *Experiment 1—Uptake of DA*

This experiment monitored the uptake of DA in crabs fed toxic clam meat for five consecutive days. The crabs were initially fed clam meats containing  $970\text{ }\mu\text{g}$  of DA (Time 0). When they were sampled postprandially at 4, 11, and 24 h, the crabs contained 155, 181, and  $260\text{ }\mu\text{g}$  of DA, respectively, that is, 16, 19, and 27% of the DA that they were fed (Table 1). The average content of DA in the HP of the crabs continued to rise to 43% ( $980\text{ }\mu\text{g}$ ) of the accumulative DA fed ( $2,270\text{ }\mu\text{g}$ ) at 48 h and 56% ( $2,160$  of  $3,830\text{ }\mu\text{g}$ ) at 96 h. The last crabs sampled were fed  $70 \pm 2.5\text{ g}$  of DA-contaminated clam meat in five feedings and achieved a mean DA-HP burden in the crabs of  $2,850\text{ }\mu\text{g}$ , or 68% of the total  $4,220\text{ }\mu\text{g}$  of DA fed (Fig. 1).

#### *Experiment 2—Depuration of DA*

This experiment compared the simultaneous depuration of DA in two groups of Dungeness crabs. One group was fed DA toxin-free clams, and the other group of crabs was starved (Fig. 2). The crabs were fed DA-containing clam meat. After feeding for 4 days, a mean concentration of  $69.5\text{ }\mu\text{g/g}$  of DA was achieved in the HP. At this point, the crabs were randomly distributed into two study groups: fed crabs were given razor clam meats containing no detectable levels of DA three times per week for 3 wk. After 7 days, the fed crabs showed a reduction in DA concentration of 38% ( $69.5\text{--}43.4\text{ }\mu\text{g}$  of DA/g), whereas the starved crabs showed only a 4% decrease in DA concentration ( $69.5\text{--}66.9\text{ }\mu\text{g}$  of DA/g). After 14 days, the DA concentration in the fed crabs decreased by 73%

TABLE 1.

Uptake of DA by Dungeness crab (*C. magister*) after periodic feedings with DA-contaminated razor clams (*S. parva*)

Feeding Times* (h)	Amount of DA Fed/Day per Crab ( $\mu\text{g}$ )	Cumulative DA ( $\mu\text{g}$ )	Sampling Times† (h)	No. of Crabs Sampled	DA in HP‡ ( $\mu\text{g}$ )	DA in HP as a % of DA Fed	Concentration of DA in HP§ ( $\mu\text{g/g}$ )
0 (initial)	970	970	0 (initial)	7	0	0	0
24	1,300	2,270	4	8	155	16	$3.5 \pm 0.6$
48	1,070	3,340	11	7	181	19	$4.7 \pm 2.0$
72	490	3,830	24	8	260	27	$5.2 \pm 1.5$
96	390	4,220	48	6	980	43	$18.4 \pm 12.4$
			72	0	NA	NA	NA
			96	8	2,160	56	$32.6 \pm 6.0$
			120	8	2,850	68	$57.5 \pm 10.3$

\* Number of hours from first feeding of toxic clams. Crabs were fed once every 24 h for 96 h.

† Except for 4 and 11 h, crabs were sampled every 24 h postprandial.

‡ The amount of DA was calculated from the weight of each HP multiplied by its DA concentration.

§ Mean  $\pm$  SD.

|| NA, not analyzed.

(69.5–19.0  $\mu\text{g}$  of DA/g), but the DA concentration in the starved crabs decreased by only 28% (69.5–49.7  $\mu\text{g}$  of DA/g). By the end of the experiment (21 days), both groups continued to depurate DA; however, the fed crabs showed the greatest decrease in DA concentration, with an 89% reduction (69.5–7.6  $\mu\text{g}$  of DA/g), whereas the DA concentration in the starved crabs decreased by 57% (69.5–29.7  $\mu\text{g}$  of DA/g) (Table 2). In each case, fed crabs had a significantly ( $p < 0.02$ ) lower mean DA concentration than starved crabs when sampled at 7, 14, and 21 days.

On Day 21 of the second experiment, the mass of HP tissue in the starved crabs was noticeably less than that in the fed crabs. The mean whole weight of the HP from starved crabs, based on com-

posited material, was 56% of the mean whole weight of the HP from fed crabs (27.2 g vs. 48.2 g). The average weight of a starved crab was more than the average weight of a fed crab (770 vs. 730 g) but was not significantly different.

A limited amount of data were collected to investigate a likely route of elimination of DA from Dungeness crabs. In the depuration experiment, DA was not detected ( $<0.5 \mu\text{g/g}$ ) in samples of hemolymph obtained from the DA-contaminated crabs at Day 1, nor from the samples taken at Day 21. Further investigation of a route of toxin elimination involved collecting fecal material. After one feeding in the first experiment, the DA concentration in feces sampled after 24 h was 2.8  $\mu\text{g/g}$ . After additional feedings, the DA content in fecal material was 2.8, 13.5, and 17.3  $\mu\text{g/g}$  at 48, 96, and 120 h, respectively.

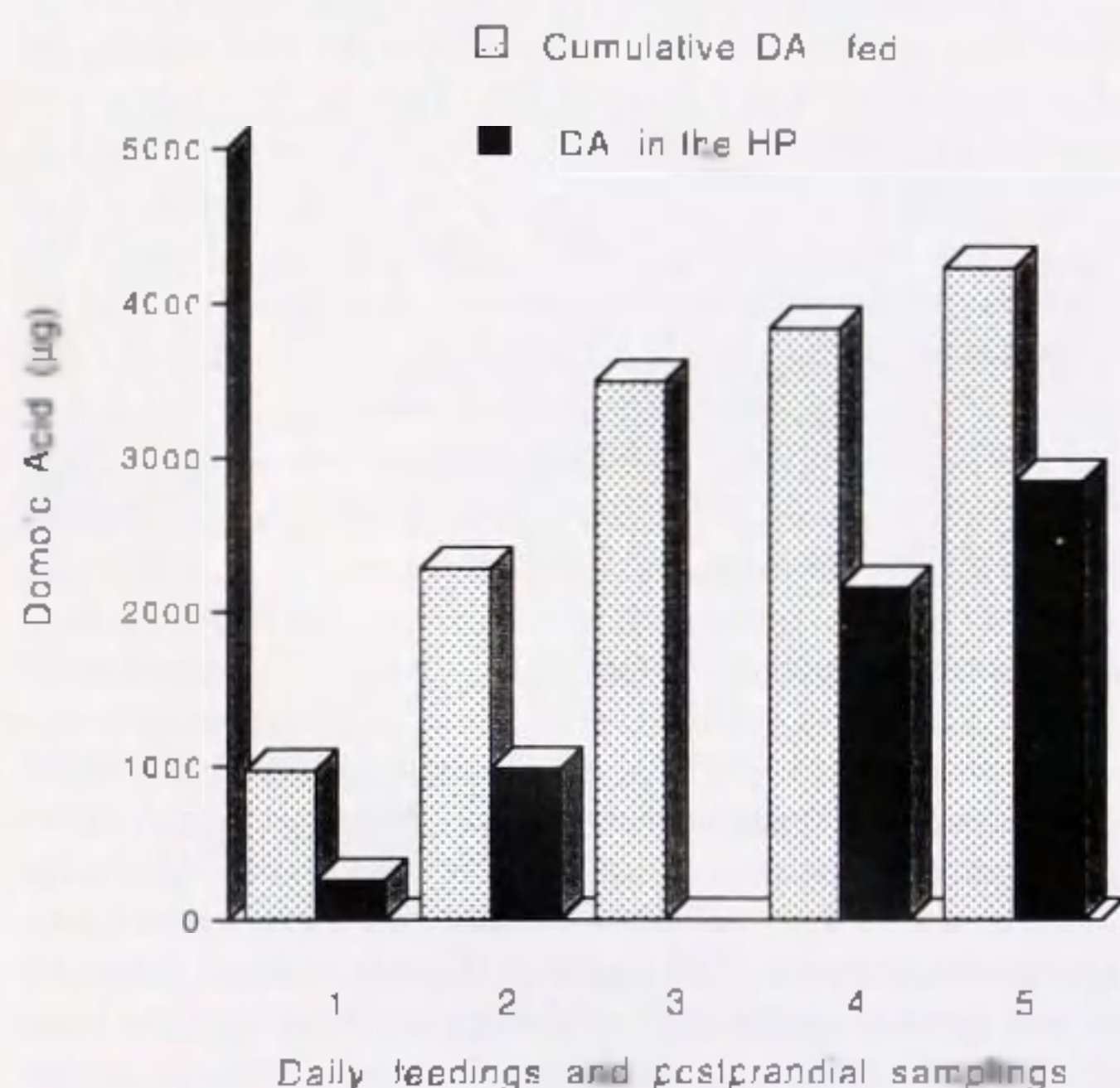


Figure 1. Summation of DA ingested by Dungeness crabs (*C. magister*) after daily feedings of toxic razor clams. Dark bars represent accumulated DA in the HP, expressed as HP burden. No samples were taken after the third feeding to maximize sample sizes after Feedings 4 and 5.

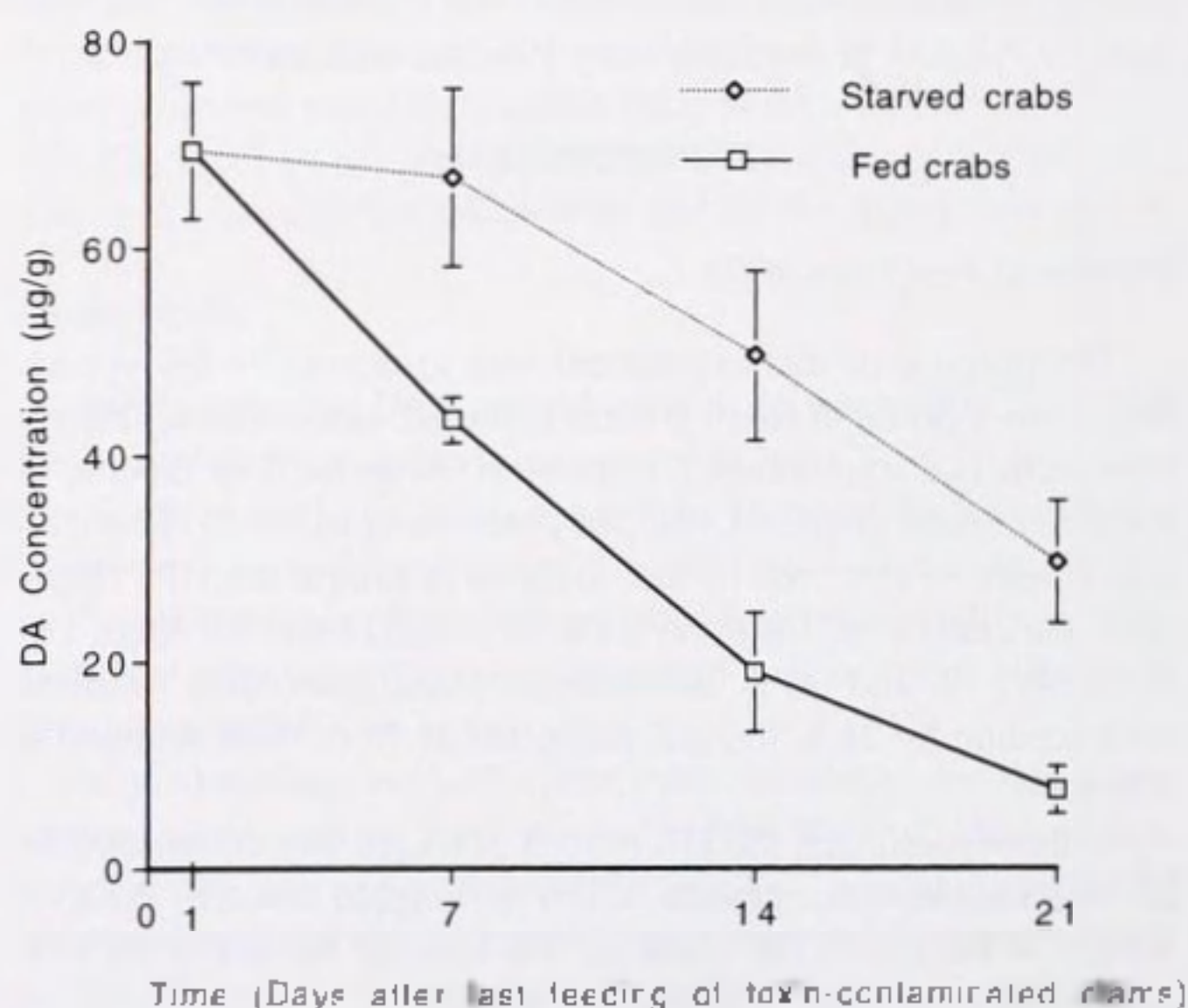


Figure 2. Effects of starvation and feeding on the concentration of DA in the HP of Dungeness crabs (*C. magister*) during depuration. Bars indicate SE,  $n = 8, 6, 7$ , and  $7$  for Days 1, 7, 14, and 21, respectively. Sampling at Day 1 represents the initial toxin level at the beginning of the experiment and occurred 24 h after the last toxic feeding.

TABLE 2.

Effects of starvation and feeding toxin-free clam meat on the depuration of DA by Dungeness crabs (*C. magister*).

Crabs	Time (days)*	n	Concentration of DA in HP ( $\mu\text{g}$ )†	SE‡	% of Initial Concentration
Fed	1	8	69.5 $\pm$ 18.6	6.6	100
	7	6	43.4 $\pm$ 6.0	2.2	62
	14	7	19.0 $\pm$ 15.4	5.8	27
	21	7	7.6 $\pm$ 6.2	2.3	11
Starved	1	8	69.5 $\pm$ 18.6	6.6	100
	7	6	66.9 $\pm$ 21.0	8.6	96
	14	7	49.7 $\pm$ 21.8	8.2	71
	21	7	29.7 $\pm$ 16.0	6.0	43

\* Number of days from last feeding of toxic clams.

† Mean  $\pm$  SD.

‡ Means with the same symbols differ significantly as compared using the Student *t*-test ( $p \leq 0.02$ ).

§ Standard error.

Crabs were fed three times per week.

## DISCUSSION

### Feeding

Razor clams are a known source of food for crabs in the wild, when available (Tegelberg 1972, Stevens et al. 1982). Crabs in this study readily consumed the commercially processed, toxic razor clams, which suggests that razor clams can be a natural vector for the toxin as well as providing a laboratory method for introducing the toxin.

Crab behavior in this study appeared to be unaffected by the ingested toxin. It is not known if there is a limit to the amount of DA the HP of a crab can retain or if there is a point at which the toxicity would begin to affect the health of the animal. In the wild, Dungeness crabs have been found with concentrations as high as 252  $\mu\text{g}$  of DA/g of HP (Chiang 1992).

Unequal feeding, which can occur from aggressive behavior in crabs and which is common with an ad libitum feeding method, was controlled by isolating the crabs individually in a net and using mechanical fingers to feed clam meat to each crab. The feeding technique yielded several other advantages over the ad libitum method. The hand-feeding method ensured that each crab ate only its portion of clam meat during a feeding and reduced the exposure time of meat to water, minimizing the possibility of leaching water-soluble DA from the clam meat. It also shortened the time period in which the crabs fed, so variation of digestion times between crabs was reduced. Furthermore, it identified crabs that were poor feeders, which were eliminated from the study.

Results reported by Wekell (1992), Wekell et al. (1994b), and Drum et al. (1993) showed that DA within the tissue of the razor clam is not uniformly distributed. For instance, the foot of the razor clam was often found to have much higher levels of DA than the siphon or body/mantle parts. Therefore, the feeding protocol described previously was adopted so that, theoretically, each crab received the same amount of DA at each feeding.

### Sampling

Although the FDA action level uses  $\mu\text{g}$  of DA/g of viscera as a unit of measure, this study focused on the HP because it is the primary organ for digestion and because it is where the bulk of the

toxin was found. Clearly, the inclusion of the rest of the visceral contents plus entrained fluids and hemolymph would dilute any toxin present. This dilution would increase the possibility of error in the estimation of DA concentration in the crabs, for the purposes of this study. To simplify sampling of the HP, but to maintain representation, HP material was removed from three anatomical areas of the organ (dorsal, ventral, lateral) and composited for analysis. Twenty-four hours postprandial was chosen as the sampling time interval because that amount of time is considered to be normal for brachyuran crabs to completely digest and assimilate a meal (Jeely and Nott 1992).

Occasionally, in this work, it was necessary to freeze samples to facilitate sample preparation and handling for chemical analysis. This technique was used without concern for loss of DA, because the toxin had been shown in the laboratory to be chemically stable in freezing, as an aqueous extract of homogenized razor clam meats (J. Wekell 1993, unpubl. data, Lund 1995) and in Dungeness crab viscera held in frozen storage for up to 1 y at  $-10^\circ\text{C}$  (Quilliam et al. 1989).

A few studies have described the feeding of toxin-contaminated materials to crabs. One such study was a limited feeding experiment where Davies (1986) fed red rock crabs (*Cancer productus*) a single ad libitum feeding of clams contaminated with PSP and sampled the crabs over a 24-h period. At 4 h, there was a low concentration of toxin in the viscera, but no toxin detectable in the muscle tissue. After 24 h, high levels of the toxin were found in the viscera, little or no toxin was found in the stomachs, and none was detected in the muscle tissues. Davies concluded that PSP toxins accumulate in the viscera, but not in the muscle tissue of the crabs, a finding similar to the results of this study.

In previous work, Foxell et al. (1979) described feeding PSP-toxic clams to a group of rock crabs (*Cancer irroratus*) for 15 wk. After dividing the crabs into two lots, one lot was starved for a week and the other was fed nontoxic clams. Analyses showed that after 1 wk, the fed crabs had 138  $\mu\text{g}$  of PSP/100 g of HP and the starved crabs had 242  $\mu\text{g}$  of PSP/100 g of HP. It is also possible that the higher concentration in the starved crabs was partly due to loss of HP mass rather than entirely from retention of the toxin. Our data also showed that feeding increased the depuration process in crabs, suggesting that depuration in Dungeness crabs may be related to metabolic processes.

In a publication by Shumway (1990), it was noted that "toxins in the gastrointestinal tract (e.g., *Mytilus*) are eliminated more readily than toxins bound in tissues (e.g., *Placopecton*, *Spisula*, and *Saxidomus*)." In DA-depuration studies by Novaczek et al. (1991, 1992), blue mussels (*M. edulis*) showed a high rate of depuration and the toxin appeared to be retained primarily in the gut lumen, as previously reported by Wright et al. (1989). Mussels with an initial concentration of 50  $\mu\text{g}$  of DA/g held under controlled laboratory conditions had only residual amounts of DA remaining after 72 h. Novaczek et al. (1992) also studied the conditions of starvation and feeding on blue mussels and found that the fed mussels consistently depurated DA more rapidly than the starved mussels. However, the differences were not considered significant. Size of animal and environmental conditions, such as water temperature, were also shown to influence the rate of depuration.

In contrast to blue mussels, razor clams distribute DA throughout their various body tissues (Wekell et al. 1992, Drum et al. 1993, Wekell et al. 1994a). Depuration studies conducted by Drum

et al. (1993) and Horner et al. (1993) found that razor clams showed little or no loss of DA after 3 mo.

Earlier work in this laboratory showed that DA accumulated only in the HP of Dungeness crab and was not found in the edible meats of body or legs (Wekell et al. 1994a, Lund 1995). Because DA did not enter the edible muscle tissue of live Dungeness crab, but was found in the HP and feces, it was reasonable to assume that depuration would occur.

In conclusion, the results of this study showed that Dungeness crabs absorb DA quickly and may eliminate some of the ingested toxin quickly as a part of the digestive process. Also, toxin accumulated in the HP with daily feedings of DA-contaminated clams and effectively depurated from the HP over a 3-wk period once the toxic feedings ceased. The depuration proceeded at a faster rate when crabs were fed than when they were starved. DA was found

in the feces of both starved and fed crabs during the experiments, which confirmed one route of toxin elimination. DA was not found in the hemolymph. Because Dungeness crabs filter hemolymph through the antennal glands, which process urine (Icely and Nott 1992), urine was considered an unlikely route of toxin elimination. It was also noted that starvation caused a loss of weight in the HP mass during 3 wk of depuration, which could result in discrepancies when interpreting toxicity levels.

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