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Feeding deterrent and toxicity effects of apo-fucoxanthinoids and phycotoxins on a marine copepod (*Tigriopus californicus*)

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Abstract Using the marine harpacticoid copepod *Tigriopus californicus*, the effects of phytoplankton feeding deterrents and toxins were differentiated and measured. Eight compounds were tested for feeding deterrence and toxicity responses: four apo-fucoxanthinoids (apo-10′-fucoxanthinal, apo-12′-fucoxanthinal, apo-12-fucoxanthinal, and apo-13′-fucoxanthinone) and four well-known phycotoxins (domoic acid, okadaic acid, microcystin-LR, and a mixture of PSP-1 toxins). Since several of these compounds exhibited both feeding deterrence and toxicity, a model was developed to deconvolute the observed toxicity response from the observed feeding deterrence response, and to classify these compounds based on the degree of toxicity and/or feeding deterrence they exhibited towards *T. californicus*. Microcystin-LR, the PSP-1 toxins, and the four apo-fucoxanthinoids behaved only as feeding deterrents at low concentrations. Okadaic acid exhibited both toxicity and feeding deterrence at low concentrations, with the threshold concentration for feeding deterrence at a lower level than the threshold concentration for toxicity. Domoic acid acted only as a toxin at low concentrations, with all decreases in feeding resulting from the death of the copepod.

Introduction

Phytoplankton produce a number of biologically active compounds, among them substances which produce either toxic effects, feeding-deterrent effects, or both. Recent work by Shaw et al. (1995a) has identified several compounds from *Phaeodactylum tricornutum*, a diatom found in tidepools, that acted as feeding deterrents against the tidepool-dwelling harpacticoid copepod *Tigriopus californicus*. These compounds were found to be feeding deterrents at low levels (2.22 to 20.2 ppm) relative to the total intracellular concentration of apo-fucoxanthinoids within the diatom cell, which ranged up to 3.34 g l^{-1} (Shaw et al. 1995a, b). Studies on these compounds (Shaw et al. 1995b) led to a need to clearly define the difference between toxic responses and feeding-deterrent responses. Some phytoplankton produce compounds, such as saxitoxin, domoic acid, and okadaic acid, which act as toxins in mammalian systems. Previous research on the paralytic shellfish poisoning (PSP) toxins produced by phytoplankton has produced conflicting results. Several researchers (Huntley et al. 1986) have suggested that the role of these compounds in nature was to act as feeding deterrents towards copepod grazers. Others (Ives 1987; Hansen 1989; Hansen et al. 1992) have shown that these compounds behave as toxins towards copepods and ciliates. Thus, more research is needed to determine if mammalian toxins are toxic to marine invertebrates or if they act only as feeding deterrents.

Feeding deterrents are compounds which bind to, and are detected by, chemoreceptors, causing the organism to cease feeding and/or avoid the compound. Chemoreceptors are generally categorized as either distant, low-concentration receptors (olfaction or smell) or contact, high-concentration receptors (gustation or taste) (Laverack 1981). However, in marine invertebrates, there is little distinction between smell and taste, as all stimuli arrive in solution and the thresholds of "taste" receptors can be quite low (Ache 1987). This simplifies research with organisms such as copepods, since one can assume that both "taste" and "smell" organs operate at similar low thresholds.

Toxins are compounds which produce observed mortality in an organism at some concentration. The mechanisms by which this observed effect occurs can be

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varied. For example, the toxin domoic acid mimics the neurotransmitter glutamate, binds to the glutamate receptor on neurons, and causes mortality by over exciting these neurons, whereas saxitoxin produces mortality by upsetting the sodium balance in cells by blocking the sodium channel. Thus, the observed effect of toxicity (death) may not readily indicate the mechanism by which the toxicity occurred.

In the present study, the lethality and feeding deterrence of four apo-fucoxanthinoid compounds produced by the diatom *Phaeodactylum tricornutum* (Shaw et al. 1995a) and four well-known phycotoxins were compared using the marine harpacticoid copepod *Tigriopus californicus* as the bioassay organism. The phycotoxins studied were domoic acid (glutamate agonist), okadaic acid (protein phosphatase inhibitor), microcystin-LR (protein phosphatase inhibitor), and a mixture of PSP (paralytic shellfish poisoning) toxins (sodium channel blockers). The data from these bioassays were used to develop a model which differentiated between toxic and feeding-deterrent responses based on the observed effects on the copepod.

Materials and methods

Phytoplankton cultures

Cultures of the diatom *Thalassiosira pseudonana* were grown as a suitable food for the copepod *Tigriopus californicus* in the feeding-deterrent bioassays. Previous studies (Shaw et al. 1995b) have shown that log-phase cultures of *T. psuedonana* do not produce feeding-deterrent effects in *T. californicus*. Unialgal cultures of *T. pseudonana* (Hustedt) Hasle and Heimdal clone 3H (NEPCC #58) were obtained from the Northeast Pacific Culture Collection (NEPCC), Department of Oceanography, University of British Columbia. Natural seawater (salinity $\approx 28‰$) was collected from West Vancouver, British Columbia at a site 100 m from shore and 15 m depth. Seawater used for culturing was filtered through activated charcoal to remove organics (Craigie and McLachlan 1964), and then filtered through a 0.8 μm Millipore filter to remove particulates. The seawater was sterilized by autoclaving. All cultures were grown using full ES enriched natural seawater (Harrison et al. 1980) at 19 °C under continuous irradiance (≈ 224 μmol m^{-2} s^{-1}), bubbled with air filtered through GF/F filters, and stirred at ≈ 60 rpm.

Copepod cultures

A culture of the harpacticoid copepod *Tigriopus californicus* was maintained to provide a continuous supply of organisms for the bioassay. *T. californicus* (Tig-1) was originally isolated from splash pools on the west coast of Vancouver Island by Dr. A.G. Lewis, University of British Columbia, in 1966 (Sullivan and Bisalputra 1980). Copepods used for the bioassay were maintained in filtered natural seawater in 1 liter Pyrex flasks at 18 °C at an irradiance of ≈ 100 μmol m^{-2} s^{-1} and a 18 h light:6 h dark photocycle. The copepods were fed either a diet of ground fish food (Wardley's Basic Fish Food for Tropical Fish) or a diet of the diatom *Thalassiosira pseudonana*.

The variability in the bioassay due to differences in the sex or life stage of the copepods was reduced by using only the adult (C6) male copepods. Differences in grazing rates were determined by measuring fecal pellet production. The water in which the copepods were incubated was quantitatively transferred to a 10 ml cylindrical settling chamber, the contents were settled for 30 min, and fecal pellets were counted at low power (100 ×) using an inverted compound microscope.

Test compounds

Semisynthetic apo-fucoxanthinoid compounds were produced from fucoxanthin as described by Shaw et al. (1995a). The following phycotoxins were available commercially: domoic acid (Sigma); okadaic acid (ICN); and PSP-1 (NRC-CNRC, Halifax, Canada). The PSP-1 mixture used in the bioassay consisted of (by mole percent) 37.1% saxitoxin-1, 34.0% neosaxitoxin-1, 23.2% gonyautoxin-2, and 5.65% gonyautoxin-3. Microcystin-LR was isolated and purified by Dr. D. E. Williams (Department of Oceanography, University of British Columbia, Vancouver, Canada).

Bioassay procedure and calculations for observed IC_{50} and LC_{50} values

A weighed amount of dried material to be bioassayed was dissolved in 1 ml of autoclaved seawater and sonicated (1 min in bath sonicator at 60 Hz) to facilitate mixing. Five ml of an exponentially growing culture of the diatom *Thalassiosira pseudonana* was added to the dissolved cell extract. Clumps were removed from the cell culture prior to use in the bioassay by filtration (forced through a 200 μm screen at a rate of ≈ 50 ml min^{-1} using a syringe; repeated four times). This mixture was placed in a glass vial (volume ≈ 7 ml) and five male C6 copepods (*Tigriopus californicus*) were added. Copepods were preconditioned in autoclaved seawater without food for 24 h prior to the bioassay. The vial was stoppered loosely with cotton wool, and placed in a wire rack. The assay was incubated for 24 h at 18 °C with an irradiance of ≈ 100 μmol m^{-2} s^{-1} and a 18 h light:6 h dark photocycle. At the end of 24 h, the number of live copepods and the number of fecal pellets in each sample were enumerated. For each compound, 5 to 7 concentrations were assayed. In each experiment, there were 5 to 6 controls, containing 5 ml of live *T. pseudonana* cells and 1 ml of autoclaved seawater.

The IC_{50} values (values at which feeding on the diatom *Thalassiosira pseudonana*, as measured by the fecal pellet production rate, is inhibited by 50%) were calculated from the data sets for each compound using a curve-fitting analysis (Shaw et al. 1995b). The result for each sample was expressed as the percentage of maximum fecal pellet production rate (calculated from the control). A curve was fit to each of the data sets using the program Sigmaplot 5.0 (Jandel Corp.). These curves were of the general equation:

$$F_o = 100 \times e^{-kc^a} \quad ,$$

where c = concentration of the bioassayed compound (μM); F_o = observed percentage maximum fecal pellet production rate, i.e. the fecal pellet production rate of the sample over that of the control ×100; and k and a are constants. This equation is based on the following assumptions: (a) $F_o \rightarrow 100$ when $c \rightarrow 0$ and (b) $F_o \rightarrow 0$ when $c \rightarrow \infty$. The IC_{50} value was then calculated by setting $F_o = 50$. The IC_{50} values of the compounds were tested for significant differences using a two-tailed t-test.

The LC_{50} values (values at which there is 50% mortality in a population of *Tigriopus californicus*) were calculated in the same manner as the IC_{50} values. The percentage survivorship was calculated for each sample. A number of the compounds tested showed no lethality in the concentration range tested. A curve was fit to each of the four data sets using the program Sigmaplot 5.0. These curves were of the general equation:

$$S_o = 100 \times e^{-kc^a} \quad ,$$

where c = the concentration of the feeding deterrent (μM); S_o = percentage of subjects which survived at the end of 24 h; and k and a are constants. This equation is based on the following assumptions: (a) $S_o \rightarrow 100$ when $c \rightarrow 0$ and (b) $S_o \rightarrow 0$ when $c \rightarrow \infty$. The LC_{50} value was then calculated by setting $S_o = 50$. The

LC_{50} values of the compounds which exhibited lethality in the bioassayed concentration ranges were tested for significant differences using a two-tailed *t*-test.

Calculations for theoretical IC_{50} values

Since mortality in the copepod population affects the observed fecal pellet production rate, it was necessary to decouple the observed mortality rates from the observed fecal pellet production rates. This produced a rate, defined as the theoretical fecal pellet production rate (F_t), which estimated the true feeding-deterrent effect of a compound in the absence of any lethal effects caused by that compound. The method used for decoupling was a linear model based on the assumption that the percentage of the population which was dead at the end of 24 h had a fecal pellet production rate of zero over the 24 h time interval. This is a very simplified model, as there was no way to predict when during the 24 h bioassay the copepods died and how many fecal pellets they produced before they died. However, within the experimental error due to the natural variability in the fecal pellet production rates of the copepods, this model was effective at decoupling mortality and feeding-deterrent effects as estimated by fecal pellet production rates. The equations for the decoupling model were as follow:

$$F_o = \frac{\text{observed fecal pellet production rate of sample}}{\text{observed fecal pellet production rate of control}} \times 100 \ ;$$

$$S_o = \frac{\text{number of copepods alive after 24 h}}{\text{total number of copepods in assay}} \times 100 \ ;$$

$$F_t = \frac{F_o}{(S_o/100)} \ .$$

The theoretical IC_{50} values were calculated using the theoretical fecal pellet production rates in the same manner that the observed IC_{50} values were calculated from the observed fecal pellet production rates. The theoretical IC_{50} values of the compounds were tested for significant differences using a two-tailed *t*-test. Differences between the observed LC_{50}, observed IC_{50}, and theoretical IC_{50} values were also tested for significance using a two-tailed *t*-test.

Results

Observed IC_{50} values

The results from the curve-fitting analyses to determine IC_{50} values are given in Table 1. At the level of significance $\alpha = 0.05$, the IC_{50} value of apo-13′-fucoxanthinone was significantly greater than the IC_{50} values of any of the other compounds. The IC_{50} value of apo-12-fucoxanthinal was significantly greater than the IC_{50} values of all the other compounds except apo-13′-fucoxanthinone and PSP-1. The IC_{50} values of apo-10′-fucoxanthinal, apo-12′-fucoxanthinal, okadaic acid, domoic acid, microcystin-LR, and PSP-1 were not significantly different.

Observed LC_{50} values

The results from the curve-fitting analyses to determine LC_{50} values are given in Table 2. At $\alpha = 0.05$, all four compounds showing lethality had significantly different LC_{50} values.

Table 1 Constants and IC_{50} values from the equation $F_o = 100 \times e^{-kc^a}$ when fitted to the data sets for each of the eight compounds bioassayed

Compound	*k*	*a*	IC_{50} (μM)	± 1 SD Range
Apo-10′-fucoxanthinal	0.499	0.197	5.26	2.10–12.6
Apo-12′-fucoxanthinal	0.310	0.390	7.88	5.98–10.3
Apo-12-fucoxanthinal	0.0559	0.914	15.7	15.3–16.2
Apo-13′-fucoxanthinone	2.97E–8	4.15	59.6	54.9–64.1
Okadaic acid	0.0490	1.25	8.38	11.2–6.00
Domoic acid	0.0524	1.10	10.4	9.19–11.8
Microcystin-LR	0.0197	1.60	9.20	10.9–7.62
PSP-1	0.179	0.448	20.5	43.6–8.86

Table 2 Constants and LC_{50} values from the equation $S_o = 100 \times e^{-kc^a}$ when fitted to the data sets for each of the eight compounds bioassayed (*NA* not applicable, highest concentrations produced no mortalities in copepods)

Compound	*k*	*a*	LC_{50} (μM)	± 1 SD Range
Apo-10′-fucoxanthinal	0.00264	1.07	180	139–230
Apo-12′-fucoxanthinal	9.38E–9	4.00	93.2	93.2–93.3
Apo-12-fucoxanthinal	NA	NA	NA	NA
Apo-13′-fucoxanthinone	NA	NA	NA	NA
Okadaic acid	0.0105	1.06	51.8	48.5–55.4
Domoic acid	0.0350	1.39	8.62	8.19–9.06
Microcystin-LR	NA	NA	NA	NA
PSP-1	NA	NA	NA	NA

Theoretical IC_{50} values

The results from the curve-fitting analyses to determine the theoretical IC_{50} values are given in Table 3. At $\alpha = 0.05$, the theoretical IC_{50} value of apo-13′-fucoxanthinone was significantly greater than the IC_{50} values of any of the other compounds. The theoretical IC_{50} value of apo-12-fucoxanthinal was significantly greater than the IC_{50} values of apo-12′-fucoxanthinal, okadaic acid, and microcystin-LR. The theoretical IC_{50} values of apo-10′-fucoxanthinal, apo-12′-fucoxanthinal, okadaic

Table 3 Constants and IC_{50} values from curve-fitting analyses of the theoretical fecal pellet production rate (*NA* not applicable)

Compound	*k*	*a*	IC_{50} (μM)	± 1 SD Range
Apo-10′-fucoxanthinal	0.475	0.144	13.8	3.98–45.0
Apo-12′-fucoxanthinal	0.338	0.335	8.57	5.76–12.5
Apo-12-fucoxanthinal	0.0559	0.914	15.7	15.3–16.2
Apo-13′-fucoxanthinone	2.97E-8	4.15	59.6	54.9–64.1
Okadaic acid	0.0480	1.18	9.59	6.74–13.1
Domoic acid	0	0	NA	NA
Microcystin-LR	0.0197	1.60	9.20	7.62–10.9
PSP-1	0.179	0.448	20.5	8.86–43.6

acid, microcystin-LR, and PSP-1 were not significantly different.

Comparison of observed LC_{50}, observed IC_{50}, and theoretical IC_{50} values

For all of the compounds which exhibited lethality, the LC_{50} values were significantly greater than either the observed or the theoretical IC_{50} values at $\alpha = 0.05$. For all the compounds except domoic acid, the observed and theoretical IC_{50} values were not significantly different at $\alpha = 0.05$.

Classification of compounds based on their feeding deterrence and toxicity activity

A model was developed to classify compounds on the basis of their feeding deterrence and toxicity. This model was based on two assumptions: (1) plots of both feeding deterrence and survivorship against concentration produce sigmoidal curves which can be fit to the equation $Y = 100e^{-kX^a}$; and (2) if F_o = observed percentage of maximum feeding rate, S_o = observed percentage survivorship, and F_t = theoretical percentage of maximum feeding rate, then $F_o = F_t \times (S_o/100)$. An example of this model is shown in Fig. 1. In this example, F_t and S_o were modeled using the following equations:

$F_t = 100e^{-3c^2}$
if $c < b$, then $S_o = 100$
if $c > b$, then $S_o = 100e^{-3(c-b)^2}$,

where c = concentration of the toxin/feeding deterrent, and $k = 3$ and $a = 2$ were arbitrarily chosen for simplicity of calculations and plotting. The value for b was varied depending on the desired overlap of the two curves. Compounds exhibiting feeding deterrence and/or toxicity were classified as one of three types depending on the degree of overlap between the observed survivorship curve and the theoretical-feeding deterrence curve.

Type I. Little or no overlap between survivorship and theoretical feeding-deterrence curves. Theoretical IC_{50} value is within ± 5% of observed IC_{50} value. Value for S_o at c = theoretical $IC_{50} \geq 95\%$. Compound exhibits only feeding-deterrent effects at low levels and only toxic effects at high levels.

Type II. Intermediate degree of overlap between survivorship and theoretical feeding-deterrence curves. Value for S_o at c = theoretical $IC_{50} < 95\%$ and $\geq 80\%$. Compound exhibits both feeding deterrent and toxic effects at all levels.

Type III. Extreme or total overlap between survivorship and theoretical feeding-deterrence curves or $F_t = 100\%$. Most or all observable feeding deterrence can be attributed to mortality. Value for S_o at c = theoretical $IC_{50} < 80\%$ and $\geq 50\%$. Compound exhibits mainly toxic effects at all levels.

This model clearly demonstrates that a single compound may have both feeding-deterrent and lethal effects. Using a simple mathematical transformation, these two effects can be quite successfully decoupled, and the compound can be classified based on its feeding-deterrent/toxic effects.

Based on the model described above, the eight bioassayed compounds were classified as follows (see Fig. 2): apo-10′-fucoxanthinal, apo-12′-fucoxanthinal, apo-12-fucoxanthinal, apo-13′-fucoxanthinone, microcystin-LR and PSP-1 were Type I; okadaic acid was Type II; and domoic acid classified as Type III.

The Type I compounds can be listed in order of decreasing feeding deterrence based on their theoretical IC_{50} values. The most effective feeding deterrents were apo-10′-fucoxanthinal, apo-12′-fucoxanthinal, microcystin-LR, and PSP-1. Apo-12-fucoxanthinal had inter-

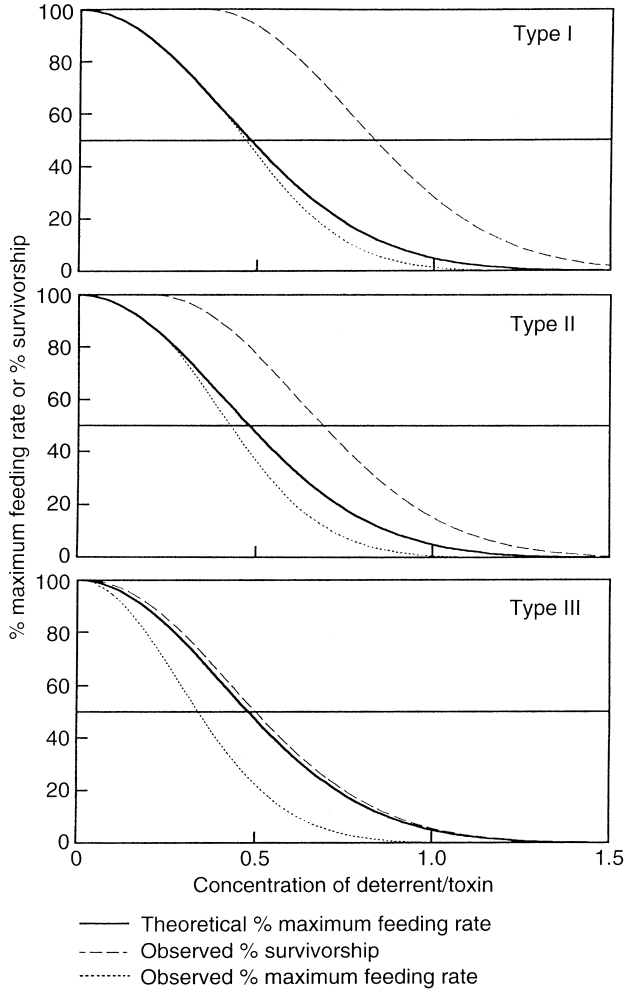

Fig. 1 Examples of classifications of feeding deterrents/toxins based on the degree of overlap between the observed survivorship curve (*dashed line*) and the theoretical feeding-deterrence curve (*solid line*) (*Type I* little overlap, theoretical IC_{50} value is within ± 5% of observed IC_{50} value; *Type II* moderate overlap, decrease in feeding due to mortality has a strong effect on observed IC_{50} value; *Type III* total overlap, decrease in feeding due to mortality can not be deconvoluted from feeding-deterrent response)

mediate feeding-deterrent activity, and apo-13′-fucoxanthinone had the least feeding deterrence. In terms of their toxicity, only apo-10′-fucoxanthinal and apo-12′-fucoxanthinal showed toxicity in the concentration ranges tested. Apo-12′-fucoxanthinal was more toxic than apo-10′-fucoxanthinal, based on their LC_{50} values.

Discussion

Research on feeding-deterrent compounds produced by phytoplankton has followed two primary approaches. On the one hand, a number of species of phytoplankton have been tested as "good" or "poor" food for various zooplankton (Stoecker et al. 1981; Egloff 1986; Huntley et al. 1986; Ward and Targett 1989). It has been postulated that "poor" food species produce chemical feeding deterrents which reduce or inhibit grazing by zooplankton. Using this approach, Shaw et al. (1995a) recently identified several compounds from a diatom that acted as feeding deterrents against a copepod. On the other hand, phycotoxins produced by phytoplankton, such as saxitoxin (Ives 1987; Hansen 1989; Hansen et al. 1992) and microcystin-LR (DeMott et al. 1991), have been tested for feeding-deterrent activity in order to understand what role these compounds play in the natural environment. These studies suggest several questions. Do phycotoxins behave as feeding deterrents in the natural environment? Do mammalian toxins have the same mechanism of action in marine invertebrates? At what point does a compound become classified as a feeding deterrent and at what point does it become classified as a toxin (since one can consider feeding to be deterred if the grazer is killed)?

In order to answer these questions, it was necessary to define toxins and feeding deterrents. In this study, a compound was considered a toxin if it produced mortality in a population within a 24 h period at a given concentration. A compound was classified as a feeding deterrent if it produced a decrease in feeding, but had no lethal effects. However, it is possible that a compound may behave as a feeding deterrent at low concentrations,

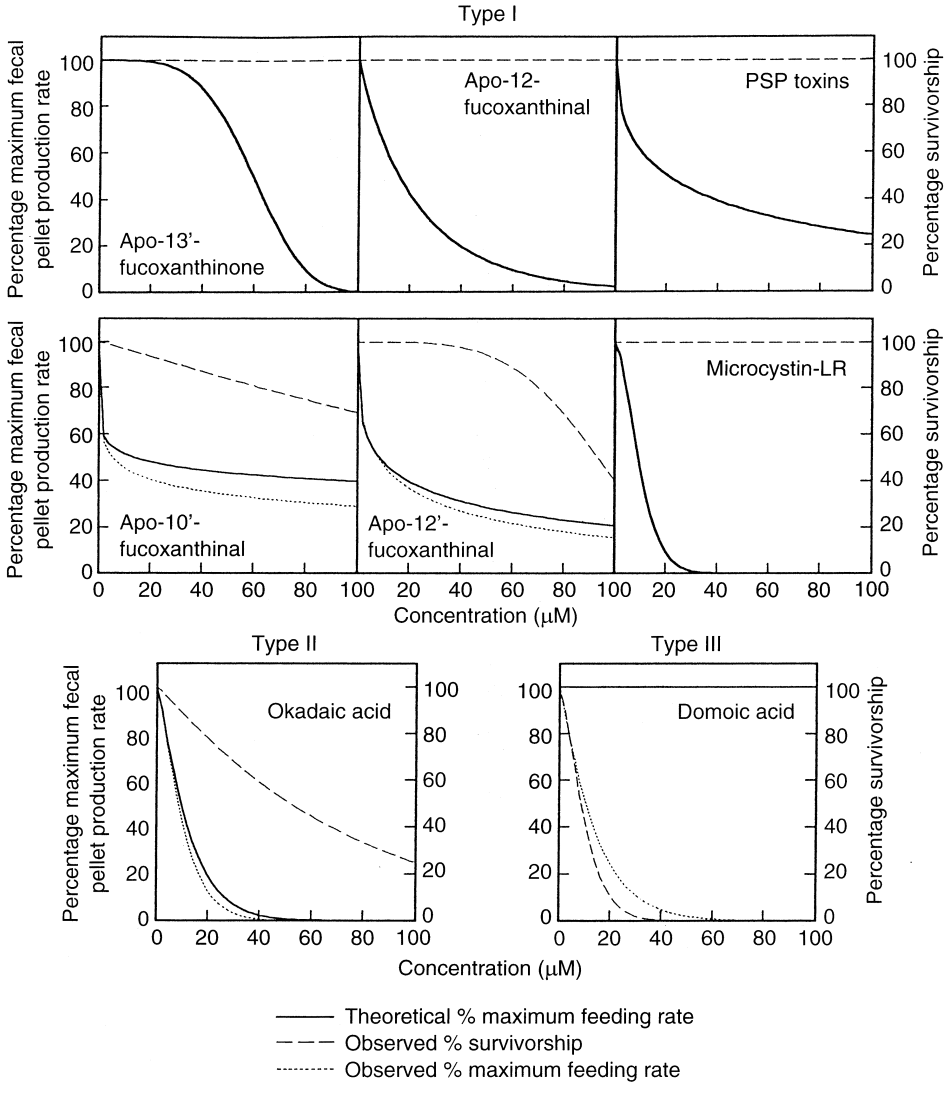

Fig. 2 Survivorship (*dashed line*), theoretical feeding-deterrence (*solid line*), and observed deterrence (*dotted line*) curves for four apo-fucoxanthinoid and four phycotoxin compounds. On several graphs, the observed feeding-deterrence curve is not visible, as it lies directly underneath the theoretical feeding-deterrence curve. Graphs are grouped according to feeding-deterrent/toxin classification, see Fig. 1

and as a toxin at much higher concentrations. At intermediate concentrations, a decrease in feeding may be due to both death of the organism and feeding inhibition. How can one deconvolute the mortality and feeding inhibition curves for compounds such as these in order to estimate the true LC_{50} and IC_{50} values? Do we classify these compounds as toxins, feeding deterrents, or both? Thus, a model was developed in this paper which classifies compounds as Type I (feeding deterrent), Type II (both feeding deterrent and toxin), or Type III (toxin) based on their observed toxicity and feeding-deterrence effects.

To understand the observed toxic or feeding-deterrent effects of a compound, one must examine the molecular basis of these responses. All chemicals eliciting a response from an organism must first interact with an organism's membranes. This interaction may be directly with the phospholipid bilayer of the membrane or with proteins embedded in the membrane. The mechanisms by which a compound interacts with a cell membrane can be grouped into three general categories (Ariëns et al. 1979; Den Otter 1981; Duncan 1981).

Direct extracellular (Fig. 3A). The compound does not cross the cell membrane, but acts directly on either the phospholipids or the proteins in the membrane to produce an effect. An example is the sodium channel blocker saxitoxin, which binds directly to the sodium channel proteins in membranes of mammalian cells, producing an ion imbalance within the cell.

Indirect intracellular (Fig. 3B). The compound interacts with the cell membrane in a manner which enables it to cross the cell membrane. The transport of the compound across the membrane may simply be passive diffusion along the concentration gradient if the compound is nonpolar, or it may be active transport by a membrane transport protein if the compound is similar to substances normally taken up by the cell. Once inside the cell, the compound then produces the observed effects by interacting with various intracellular components.

Indirect, receptor-mediated (Fig. 3C). The compound does not cross the cell membrane, but binds directly to a receptor protein in the membrane. This binding activates the receptor, producing a generator potential across the membrane which triggers an intracellular chain of reactions, leading to the observed effect (Ariëns et al. 1979; Den Otter 1981). Two examples of this are the binding of a neurotransmitter (e.g. domoic acid in mammalian systems) to a receptor on a neuron, and the binding of a "sensed" compound to a chemoreceptor (as in taste and smell).

Thus, although the biochemistry of the cell response is quite different for each of these mechanisms, all three are initiated by an interaction with the surface of the cell. In addition, a given compound may act by more than one of these mechanisms. As a result, it may be difficult to determine which mechanism is producing the observed effect in an organism. However, it may still be possible to distinguish between different observed effects

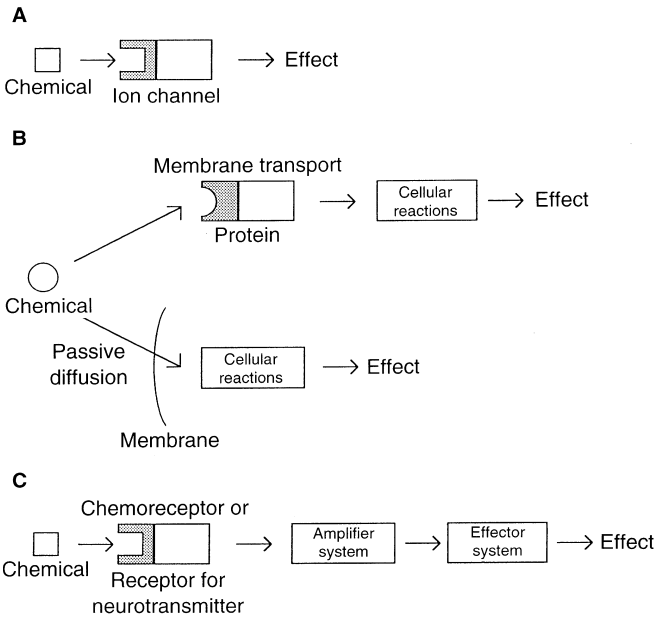

Fig. 3 Three general models showing mechanisms by which a compound may interact with a cell membrane: **A** direct extracellular mechanism; **B** indirect intracellular mechanism; **C** indirect receptor-mediated mechanism

(e.g. toxicity and feeding deterrence), which may give some clues about the cellular mechanisms.

We can now attempt to describe the various categories from the model developed in the present paper in terms of molecular mechansisms. The major mechanism responsible for the observed effects in Type I compounds at low concentrations is probably an indirect, receptor-mediated mechanism, such as would be expected for chemoreception. Since the response to the compound binding to the chemoreceptor is amplified, the copepod has the ability to detect and respond to Type I compounds at very low thresholds. The four apo-fucoxanthinoid compounds may be detected by a single, generalist chemoreceptor which is broadly "tuned" to compounds with similar chemical structures (Finger and Silver 1987). However, there were probably at least two other types of chemoreceptors responsible for the activity observed in our study, as microcystin-LR and the PSP-1 compounds are chemically very different from each other and from the apo-fucoxanthinoids.

The Type II compound, okadaic acid, showed both toxicity and feeding deterrence at low threshold concentrations. The feeding-deterrence response was probably due to the activation of chemoreceptors as described above. Okadaic acid is a phosphatase inhibitor. However, this activity can only be expressed after the compound has crossed the cell membrane (indirect intracellular mechanism). Microcystin-LR, another phosphatase inhibitor, did not show toxicity at a low concentration [which contradicts a study by DeMott et al. (1991), showing microcystin-LR toxicity in the freshwater copepod *Diaptomus birgei*, but supports recent research by Jungmann and Benndorf (1994),

showing no correlation between concentrations of microcystins and toxicity to *Daphnia pulicaria*]. Thus, it seems that okadaic acid, unlike microcystin-LR, is able to cross cell membranes. This may be due to the fact that okadaic acid is less polar than microcystin-LR, and may be able to passively diffuse across a membrane, or that okadaic acid may be binding to a membrane transport protein and thus be actively transported across the membrane.

Domoic acid was classified as a Type III compound. It did not appear to act as a feeding deterrent, but was toxic to the copepod at very low concentrations. In mammalian systems, domoic acid acts as a glutamate agonist, causing damage to neurons by binding to the glutamate receptor and overexciting the neurons. Thus, the toxicity of domoic acid is caused by an indirect, receptor-mediated mechanism. This mechanism involves amplification of the initial response to receptor binding, and therefore has a low threshold concentration for toxicity by domoic acid. The experimental results for the effects of domoic acid on *Tigriopus californicus* suggest that the mechanism of action is the same in this copepod as it is in mammalian systems. The LC_{50} value for domoic acid (8.62 μM) was in the same range as the IC_{50} values for Type I compounds (8.57 to 59.6 μM). This is not surprising, since the mechanism responsible for chemoreception (measured by IC_{50} values) is probably also an indirect, receptor-mediated mechanism with a threshold of detection in the same range as for domoic acid toxicity.

In summary, it appears that the observed response of the copepod *Tigriopus californicus* to certain dissolved chemicals is dependent upon the mechanism by which those chemicals interact with receptors and cell membranes in the organism. A model has been developed to classify feeding-deterrent and toxicity responses based on the observed effects of various compounds on this copepod. Since toxins and feeding deterrents have different impacts on food webs, this model will play an important role in determining the ecological significance of biologically active compounds produced by phytoplankton in the natural environment. Compounds such as the apo-fucoxanthinoids, microcystin-LR, and the PSP-1 toxins are detected by a chemoreceptor, causing the copepod to cease feeding, thus avoiding potential toxicity of the compounds. Domoic acid, however, does not appear to be detected and avoided, and probably acts as a neurotoxin. Okadaic acid has both feeding-deterrent and toxicity responses, with the chemoreceptor response having a lower threshold concentration than the toxicity response. This compound may well be avoided by the copepod in a natural setting where the copepod is free to swim away from the zone of highest concentration.

Therefore, it appears that at least three of the well-known types of phycotoxins, microcystin-LR, PSP-1 toxins, and okadaic acid, act as feeding deterrents for *Tigriopus californicus* at low concentrations. The feeding deterrence of the PSP toxins agrees with previous work done by Hansen et al. (1992). Thus, it appears that phycotoxins may play a role as feeding deterrents in the natural environment, providing some protection against grazers. Domoic acid and okadaic acid are toxic to *T. californicus*. Domoic acid may act as a glutamate agonist. However, further research is required to verify if the modes of action for both okadaic acid and domoic acid are the same as for mammalian systems.

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